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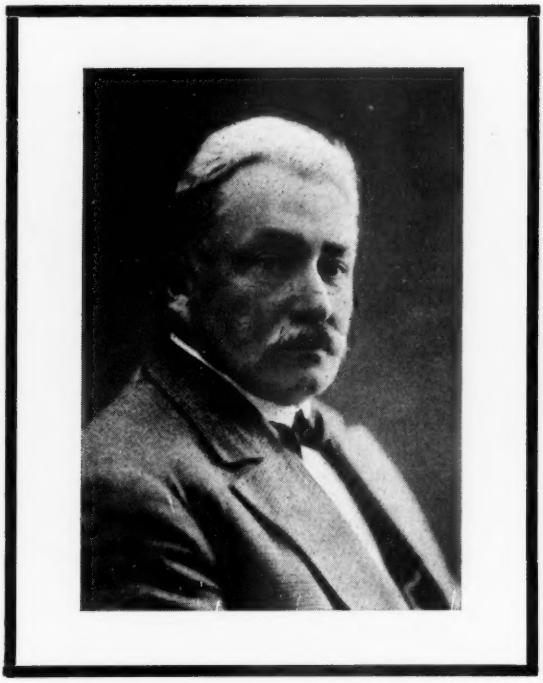

WERNER OSWALD RENKONEN

SEPT. 6, 1872 — SEPT. 7, 1951

Werner Oswald Renkonen (formerly Streng), Archiater, Emeritus Professor of Bacteriology and Serology in the University of Helsinki, died of pulmonary cancer in Helsinki on Sept. 7, 1951, the day following his seventy-ninth birthday. He was the Nestor of the Finnish medical profession, a distinguished man of research and a fine teacher, a practitioner of the first rank, and a respected and admired personality.

Born in Heinola, W. O. Renkonen went to school at Mikkeli Lyceum and in 1890 entered the Medical Faculty of Helsinki University. After graduating in 1895, he became licentiate in medicine in 1900 and M.D. in 1902. In 1903 he was appointed Lecturer in bacteriology at the University of Helsinki and in 1911 Associate Professor. In 1921 he became the first holder of the regular chair of bacteriology and serology in the same university. He retired in 1942. He was Vice-Dean of the Medical Faculty between 1927 and 1930, and Dean between 1930 and 1936. He was also Head

of the College of Gymnastics in the University of Helsinki from 1928 to 1936 and Patron of the corporation of students from the district of Savo from 1923 to 1925.

On many committees appointed by the University of Helsinki and the Board of Health, in the Finnish Academy of Science and Letters, and on the executive committee of the Association for the Prevention of Tuberculosis, Renkonen contributed in a valuable way towards the promotion of research and public health. He was on the committee appointed to elaborate plans for a State serological institute, which was to become an important centre for bacteriological and serological diagnosis and for the preparation of vaccines, and a Pasteur laboratory for the preparation of rabies vaccine and for the treatment of rabies. Renkonen was also chairman of the State committee which controls the preparation of sera and vaccines, and of another State committee appointed to make plans for an institute of public health. It was particularly the latter of these committees that gave him an opportunity for valuable pioneer work for public health. Renkonen was also chairman of a committee appointed to reorganise the University College of Gymnastics, and on committees appointed for the rebuilding of the premises of the Departments of Medical Chemistry and Pharmacology and for the organising of a system of central hospitals. Renkonen was also one of those who elaborated the plans for the research and educational programme of the new university at Turku. Later, he acted as medical adviser in a committee which was to draw plans for the foundation of a Department of Serology and Bacteriology in the same university (the University of Turku).

Renkonen's activity as a practitioner specialising in internal and pulmonary diseases is an important aspect in his medical career. He was chief physician of the Pohja Life Insurance Company from 1928 to 1945. The title of Archiater was conferred on him in 1943.

Renkonen was an active member in numerous medical societies; in many of these his contribution has been of primary importance. As an enthusiastic champion of the rights of the Finnish-speaking population he joined the Finnish Medical Society Duodecim soon after it had been founded in the 1880's. All through his life he contributed in many ways to the welfare of this society, which remained near his heart until the end. He was the Hon. Treasurer of the society from 1898 to 1900 and its chairman from 1911 to

1914. He was the editor of the medical journal of the society, also called *Duodecim*, from 1912 to 1917. He was elected an honorary member of the society in 1931. Renkonen's death is also heavily felt among the members of the Finnish Pathological Association and the Scandinavian Pathological Society. These societies came near his basic interests in medical science. He was an honorary member of both societies and chairman of the Finnish Pathological Association. He attended numerous Scandinavian pathological congresses and presided in some of them. Renkonen was also a member in the Helsinki Medical Society (chairman from 1917 to 1918), the Finnish Dermatological Society (chairman in 1924), and the Finnish Society of Physicians (an honorary member).

The most important aspect in Renkonen's long career was, however, his contribution in the field of medical research. He was also an academic teacher of the best type. As the first holder of the chair of serology and bacteriology in the University of Helsinki he came to be a pioneer in Finnish work on these fields of medical research. His achievements won for him an esteemed place among the specialists of these fields. His publications deal, for instance, with animal experiments concerning the effect of bacteria and toxins on the function of the organism, conglutination, serological syphilis reactions, and blood groups. He became famous all over the world through his studies on the anthropology of the blood groups. Thanks to his work, the Finnish people is one of the best studied in this respect. A fine disposition, a careful performance, and an extremely critical assessment of the results are characteristic of Renkonen's scientific work, which has very materially contributed to the esteem in which Finnish medical research is held in the world. Renkonen was a profound but at the same time clear thinker, a full-blooded representative of a scientist of his time. During long visits to leading European laboratories he acquired a thorough knowledge of his field and established lasting contacts with leading authorities.

Renkonen was an indefatigable scientific teacher, who never spared his time or pains in initiating his pupils into the secrets of medical research. A true philosopher, a sceptic but at the same time an idealist, he was a reliable guide to medical thought. The present wide and keen interest in serology and bacteriology in Finland, which is to be considered essentially a result of Renkonen's activity

as a scientific teacher, is a real wonder considering the poor economic conditions under which theoretical medical research is carried on in Finland.

A man of sharp wit, friendly character, and a rare sense of humour, Renkonen made a large number of friends. In his own home he was an excellent host. He was a welcome and respected guest at the festival meetings of the student corporations, which he seldom was the first to leave. On his sixtieth birthday in 1932 his pupils and friends had a portrait painted of him, and a number of *Acta Duodecim* was also dedicated to him. Another festival volume, this time a number of *Annales Medicinae Experimentalis et Biologiae Fenniae*, was dedicated to him on his seventy-fifth birthday in 1947.

His cheerful mind and his strong physique helped him to carry the weight of the years surprisingly well. A more harmonious aging is seldom seen. The heavy wars in which his country became involved he went through in an exemplary manner. He gave all his energy to war-time laboratory and blood donor service. But his energy was by no means used up when the peace came with its hard terms. Indefatigable, he was among the first to organise rehabilitation work. During his last years Renkonen took a keen interest in gerontology, above all by taking an active part in the activity of the Finnish Gerontological Society. His last illness did not repress his mental energy nor his belief in the richness and significance of life. With a wonderful tranquillity of mind he prepared for what awaits every human being at the end.

In Renkonen Finland lost her most prominent medical personality of the present day. This loss is particularly heavy to his family and his wide circle of friends, pupils, and colleagues. Renkonen's work and his cheerful, engaging personality will be long remembered.

Turku, 31 Oct., 1951.

Eero Mustakallio

AUS DEM SEROBAKTERIOLOGISCHEN INSTITUT DER UNIVERSITÄT HELSINKI

ZUR KENNTNIS DER TITERWERTE BEI DEN ISOAGGLUTINATIONSREAKTIONEN UND DEREN VERWENDUNG

I. ÜBER DEN VERSUCHSFEHLER

Von

OSV. RENKONEN (STRENG) und ESKO SUOMALAINEN

Die Möglichkeit, konstante Titerwerte bei den Isoagglutinationsreaktionen, bei welchen mitwirkende Faktoren: die Empfindlichkeit der Blutkörperchen, die agglutinierende Stärke der Sera und das Milieu (Technik und Methodik), alle von variierender Grösse sind, ist öfters diskutiert worden. Eine Übereinstimmung der Auffassungen auf diesem Gebiete ist nicht erreicht worden. Wir verweisen betreffs der Literatur auf die Handbücher, auf die Protokolle der im Anschluss an den ersten allgemeinen Mikrobiologenkongress in Paris im Jahre 1930 gehaltenen Konferenz der Isoagglutinationsforscher sowie auf die Arbeit von Osv. Renkonen (Streng) (1).

Es ist allgemein anerkannt, dass steril entnommenes Serum, gegen Austrocknung geschützt und im Dunkeln im Eisschrank aufbewahrt, ziemlich lange (mehrere Monate hindurch) seine Stärke unverändert beibehält. Weiter ist man sich wohl auch darüber einig, dass sich die Empfindlichkeit der Blutkörperchen nach der Entnahme trotz sachgemässer Aufbewahrung ziemlich schnell verändern kann. Man müsste somit mit sterilen Sera und möglichst frisch entnommenen Blutkörperchen arbeiten. Die Frage, ob es notwendig ist, dass nur am Titrierungstage entnommene Blutkörperchen verwendet werden, ist nicht ganz klar. Wie wir zeigen werden, sind ein paar Tage alte steril aufbewahrte Blutkörperchen unter gewissen Bedingungen noch verwendungstauglich. Die durch

die Aufbewahrung zustandekommenden Veränderungen der Empfindlichkeit der Blutkörperchen und die davon herrührenden Fehler der Titerwerte sind offenbar bedeutend kleiner als diejenigen, welche darauf beruhen, dass trotz einer sonst durchaus identischen Technik eine identische Ablesung der Titerwerte derselben Sera an verschiedenen Tagen schwierig oder sogar unmöglich fehlerfrei durchführbar ist. Schon an ein und demselben Tage ausgeführte Isoagglutinationstitrierungen sind ohne grosse, sogar 50%ige Fehler schwierig abzulesen und miteinander zu vergleichen. Um so schwieriger wird trotz gebührenden Kontrollen und Einhaltung aller Vorsichtsmassregeln einer gleichmässigen Technik die identische Ablesung, wenn Titerwerte über längere Zeiten (Monate und Jahre), gesammelt werden. Nur durch grosse Serien und Mittelwertsberechnungen lässt sich die Bedeutung der technischen Fehler allerlei Art beurteilen und ausschalten.

In der vorliegenden Arbeit soll zunächst Näheres über unsere Erfahrungen betreffs der Versuchsfehler berichtet werden. — Ganz zuerst jedoch einige Worte über das Blut in vivo.

VERÄNDERUNGEN DES BLUTES IM LEBENDEN ORGANISMUS

Die agglutinierende Stärke des *Serums* steigt beim Individuum, wie Thomsen und Kettel (2) gezeigt haben, von der Geburt an ziemlich schnell. Der Mittelwert erreichte nach den genannten Autoren in dänischem Material sein Maximum in der Altersgruppe von 5 bis 10 Jahren. Danach nimmt der Mittelwert der Serumstärke schon in der Altersgruppe von 10 bis 20 Jahren etwas ab, so dass in dieser Altersgruppe der Mittelwert z.B. für Anti-A in O-Sera von 1: 386 bei den 5—10jährigen auf 1: 332 gesunken war. Später erfolgt die Abnahme langsamer. In der Altersgruppe von 50 bis 60 Jahren war der Mittelwert noch 1: 174, was ungefähr der halben Serumstärke bei den 10- bis 20jährigen entspricht, und erst bei den 90- bis 100jährigen sinkt der Mittelwert der agglutinierenden Kraft wieder auf den Wert des Säuglingsalters zurück. Die individuellen Verhältnisse spiegeln sich naturgemäss in den Mittelwerten der 10jahrsgruppen nicht wider. Die Tatsache, dass man bei jungen gesunden Individuen im Alter von 10 bis 20 Jahren manchmal ganz niedrige Titerwerte finden kann, und anderseits bei Greisen im Alter von 80 bis 100 Jahren im Gegenteil hin und wieder

auch starke Reaktionen vorkommen können, zeigt, wie kompliziert die Frage ist. Vergleiche auch mit den Arbeiten (3) und (4).

Die von uns untersuchten Individuen waren junge Blutspenderaspiranten meistens im Alter von 20 bis 30 Jahren, vorwiegend Frauen. Manchmal befanden sich die Untersuchten auch im Alter von 30 bis 40 Jahren, manchmal auch ein paar Jahre unter 20. Unser Material ist somit auf ungefähr dieselbe Altersgruppe beschränkt und betrifft also weder ganz junge noch ältere Personen, sondern nur Erwachsene in jungen Jahren. Die individuellen Serumtiterwerte variieren der Literatur gemäss von 1: 1 bis etwa 1: 2000. Die Differenzen der Mittelwerte der 10jährigen Altersgruppen bei Erwachsenen von 20 bis 60 Jahren waren bei Thomsen und Kettel (2) kleiner als eine Verdünnungsstufe. Im anthropologischen Alter (von 20 bis 50 Jahren) waren die Differenzen noch kleiner (2). Bei interkurrenten akuten Krankheiten haben sich keine nennenswerten Veränderungen der Titerstärke bei dem einzelnen Individuum ergeben (5, 6, 7, u.a.). Doch ist hervorzuheben, dass die ganze Frage von eventuellen Schwankungen der Titerwerte bei chronischen Krankheiten heute noch als ziemlich offen zu betrachten ist. Es gibt überhaupt nur wenige Untersuchungen, wo die Titerwerte bei den verschiedenen Krankheitsstadien der Individuen miteinander methodisch verglichen worden wären. Inwieweit sich die Mittelwerte der Gesunden bei anderen Völkern mit den dänischen decken, weiss man nicht. Wir haben jedenfalls in Finnland für das anthropologische Alter andere Zahlen gefunden. Vergleiche auch Schiff (8).

Die Reaktionsstärke beruht aber nicht nur auf der Stärke des Serums, sondern wie bekannt auch auf der Empfindlichkeit der *Blutkörperchen*. Diese steigt beim lebenden Individuum wie die Serumstärke von der Geburt an und erreicht erst bei etwa 20 Jahren ihr Maximum (2, 9, u.a.). Man hat angenommen, dass die Empfindlichkeit der Blutkörperchen danach das ganze Leben hindurch unverändert bleibt (2, 9). Mit den obenangeführten, die Serumstärke in verschiedenen Altersgruppen betreffenden Untersuchungen vergleichbare grössere Untersuchungsserien betreffs der Empfindlichkeit der Blutkörperchen in verschiedenen Altersgruppen haben Thomsen und Kettel unseres Wissens jedoch nicht veröffentlicht. Ihr Befund betreffs ganz frischer Blutkörperchen, dass in den höchsten Altersgruppen (90 bis 100 Jahre und mehr) eine ungefähr ähnliche Empfindlichkeit der Blutkörperchen wie

in jüngeren Jahren vorhanden ist, spricht entschieden für eine gewisse Konstanz der Empfindlichkeit der Blutkörperchen auch im anthropologischen Alter. Ebenso weist die gefundene Tatsache (2), dass bei 85% der in Dänemark mit frischen Blutkörperchen ausgeführten Titrierungsserien die Empfindlichkeit der Blutkörperchen, mit demselben Serum gemessen, identisch war, in dieselbe Richtung. Doch sind nach Thomsen und Kettel (2) immerhin auch bei Verwendung von ganz frisch entnommenen Blutkörperchen in 15% kleinere individuelle Unterschiede der Empfindlichkeit der Blutkörperchen zu verzeichnen.

DIE MÖGLICHEN FEHLERQUELLEN UNSERER TECHNIK.

Unsere Arbeit wurde während des Krieges ausgeführt. Betreffs unserer Technik, die früher (1) beschrieben worden ist, möchten wir deshalb noch hinzufügen, dass wir während unserer fast dreijährigen Untersuchung an jedem Titrierungstag eine Kontrolltitrierung mit Serum von derselben Person (Fräulein J—n) angestellt haben. Das Serum von Fräulein J—n wurde nach verschieden langen Zeiten entnommen und steril im Eisschrank aufbewahrt.[1]

Die Resultate der Titrierungen mit dem Kontrollserum von Fräulein J—n sind in der Tab. I zusammengestellt.

Ohne Zweifel liegt der Mittelwert für Anti-A_1 im Kontrollserum »J—n» bei einer Verdünnung zwischen 1/32 und 1/64. Der Mittelwert für Anti-B war ungefähr derselbe. Das Serum von Fräulein J—n (1 bis 12 Tage steril aufbewahrt) hat (siehe Tab. III) auch mit ganz frischen A_1- und B-Blutkörperchen gemessen ungefähr dasselbe Resultat gegeben. Wir glauben daher, dass unsere Resultate mit anderen Sera, obwohl wir die Sera nicht alle Tage mit am gleichen Tage entnommenen Blutkörperchen austitriert haben, doch richtig gewesen sind. Auf jeden Fall enthalten unsere Resultate nicht so grosse Fehler, wie solche bei verschiedenartiger Ablesung auftreten können.

Um diese Frage noch weiterhin zu beleuchten, haben wir die Differenzen zwischen den mit dem Serum von Fräulein J—n erhal-

[1] Bei der Ablesung der Titrierungen sind uns die damaligen Assistenten des Serobakteriologischen Instituts zeitweise behilflich gewesen. Unter ihnen möchten wir besonders den Herren Doktoren R. Koulumies, E. Uroma und K. Penttinen danken. Auch Frau Elsa Vuorinen, die uns bei den mathematischen Ausrechnungen geholfen hat, möchten wir unseren besten Dank zum Ausdruck bringen.

TABELLE I

O-SERUM VON FRÄULEIN J—N, AN VERSCHIEDENEN TAGEN WÄHREND DER JAHRE 1941—1943 AUSTITRIERT. IN DER TABELLE SIND NUR DIE MIT A_1- UND B-BLUTKÖRPERCHEN ERHALTENEN RESULTATE ANGEGEBEN, DIE TITRIERUNGEN MIT A_2-BLUTKÖRPERCHEN SIND WEGEN IHRER GERINGEN ANZAHL WEGGELASSEN. DIE ALS INDIKATOR BENUTZTEN BLUTKÖRPERCHEN WAREN TEILWEISE FRISCH ENTNOMMEN ODER LEGE ARTIS STERIL HÖCHSTENS EIN PAAR TAGE AUFBEWAHRT.

O Anti-A_1

Die Serumverdünnungen	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	Summe	die Mittelwerte der Titer
Blutkörperchen von											
Frl. H—a, abs. Anzahl	—	—	—	1	13	42	57	8	—	121	51.50
%				0.8 %	10.8 %	34.7 %	47.1 %	6.6 %			
Frl. N—i, abs. Anzahl	—	—	—	—	5	19	36	4	—	64	54.75
%					7.8 %	29.7 %	56.3 %	6.3 %			
Frl. K—a, abs. Anzahl	—	—	—	—	3	9	10	1	—	23	48.0
Drei verschiedene Blutkörperspender	—	—	—	—	3	4	14	3	—	24	
Im ganzen abs. Anzahl	—	—	—	1	24	74	117	16	—	232	53.0
%				0.4 %	10.4 %	31.9 %	50.4 %	6.9 %			
						82.3 %					

O Anti-B

Die Serumverdünnungen	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	Summe	die Mittelwerte der Titer
Blutkörperchen von Frau K—o, abs. Anzahl	—	—	2	2	1	38	59	3	—	105	51.58
%			1.9 %	1.9 %	0.95 %	36.2 %	56.2 %	2.85 %			
Frl. Sch—n, abs. Anzahl	—	—	—	5	8	37	50	2	1	103	49.17
%				4,9 %	7.8 %	35.9 %	48.5 %	1.9 %	1 %		
Drei verschiedene Blutkörperspender	—	—	—	—	—	10	14	—	—	24	
Im ganzen abs. Anzahl	—	—	2	7	9	85	123	5	1	232	50.41
%			0.9 %	3.0 %	3.9 %	36.6 %	53.0 %	2.2 %	0.4 %		
						89.6 %					

$$\frac{\text{O Anti-A}_1}{\text{O Anti-B}} = \frac{53.0}{50.41} = 1.05$$

tenen Werten folgendermassen paarweise miteinander verglichen. Der Wert des ersten Versuchstages wurde demjenigen des zweiten Tages, der Wert des zweiten Tages demjenigen des dritten Tages, usw., gegenübergestellt und die Differenzen notiert. Waren die Titerwerte zweier aufeinanderfolgender Tage gleich stark, so wurden die Differenzen mit 0 bezeichnet. Trug der Grenzwert bei derselben Verdünnung in den Protokollen an einem Tage das Zeichen +, am anderen ±, so wurde die Differenz mit ½ bezeichnet. Erstreckte sich die Differenz über eine, zwei oder mehrere Verdünnungsstufen, so wurde sie beziehungsweise mit 1, 2, 3 usw. bezeichnet. Wir erhielten auf diese Weise eine ganze Skala von Differenzen, 0, ½, 1, 1½, 2, 2½ usw. Jeder Grenzwert wurde somit zweimal berücksichtigt: Titrierung am 1. Tag mit Titrierung am 2. Tag, 2. mit 3., 3. mit 4. usw., der letzte Wert, Titrierung 232, wurde mit dem ersten, also mit Titrierung 1., verglichen. Die Tabelle II zeigt die Resultate.

TABELLE II

SERUM VON FRÄULEIN J—N. DIE DIFFERENZEN DER TITERWERTE DER EINANDER GEGENÜBERGESTELLTEN TITRIERUNGEN.

	Differenzen in Stufen															
	0		½	1	1½	2	2½	3	3½	4	4½	usw.				
0 Anti-A_1																
Anzahl	77	74	50	17	7	4		2	1	—	—		= 232 »Paare»			
%	86.6 %			13.4 %												
0 Anti-B																
Anzahl	64	73	61	19	7	2		1	2	3	—		= 232 »Paare»			
%	85.3 %			14.7 %												

Die beobachteten Differenzen waren somit ziemlich gross, und auf jeden Fall waren sie hier grösser als diejenigen, die man findet, wenn dasselbe, verschieden lange Zeit aufbewahrte Serum mit frischen Blutkörperchen desselben Individuums *am gleichen Tage* austitriert wird. Siehe die Tabelle III.

Auch hier haben wir Differenzen notiert, sie waren aber bedeutend kleiner als in Tabelle II. Hier betrugen die Differenzen in 98.2% höchstens eine Stufe, dort war der entsprechende Prozentsatz 85.3% bzw. 86.6%. Des Vergleichs halber möchten wir noch die folgende Tabelle IV wiedergeben, aus welcher hervorgeht, dass

TABELLE III

SERUM VON FRÄULEIN J—N, MIT FRISCH ENTNOMMENEN BLUTKÖRPERCHEN VON FÜNF PERSONEN AUSTITRIERT. DIE FÜNF PERSONEN WAREN 2 A_1-, 2 B- UND 1 A_2-INDIVIDUEN. DAS SERUM VON FRL. J—N AN VERSCHIEDENEN TAGEN ENTNOMMEN UND VERSCHIEDEN LANGE ZEIT (3, 5, 7, 9, 11 UND 12 TAGE) LEGE ARTIS AUFBEWAHRT. DIE EMPFINDLICHKEIT DER VERSCHIEDENEN BLUTKÖRPERCHEN PAARWEISE MITEINANDER VERGLICHEN. ABLESUNGEN AM GLEICHEN TAGE,

Differenzen in Stufen

O Anti-A_1 und O Anti-B zusammen	0	½	1	1½	2	2½	3
Anzahl	27	21	8	1	—	—	—
%	98.2 %			1.8 %			

TABELLE IV

10 VERSCHIEDENE O-SERA, MIT A_1-BLUT VON ZWEI VERSCHIEDENEN PERSONEN, B-BLUT VON ZWEI VERSCHIEDENEN PERSONEN UND A_2-BLUT VON EINER FÜNFTEN PERSON GEMESSEN.

1.

Die Empfindlichkeit frisch entnommener Blutkörperchen wurde an zwei verschiedenen Tagen mit jedem von den 10 Sera untersucht. Die Blutspender die obengenannten. O Anti-A_1 und O Anti-B zusammen behandelt (ebenso in 2.—4.).

Differenzen in Stufen

	0	½	1	1½	2	2½	3
Anzahl	15	21	7	3	3	—	—
%	88 %			12 %			

2.

Frisch entnommene Blutkörperchen wurden an zwei verschiedenen Tagen mit zwei Tage alten lege artis aufbewahrten Blutkörperchen verglichen. Die Sera die obengenannten.

Differenzen in Stufen

	0	½	1	1½	2	2½	3
Anzahl	22	51	12	8	6	1	—
%	85 %			15 %			

3.

Frisch entnommene Blutkörperchen wurden an zwei verschiedenen Tagen mit vier Tage alten lege artis aufbewahrten Blutkörperchen verglichen. Die Sera die obengenannten.

Differenzen in Stufen

	0	½	1	1½	2	2½	3
Anzahl	23	40	21	14	2	—	—
%	84 %			16 %			

4.

Zwei und vier Tage lege artis aufbewahrte Blutkörperchen wurden an zwei verschiedenen Tagen unter sich verglichen. Die Sera dieselben wie oben.

	Differenzen in Stufen						
	0	½	1	1½	2	2½	3
Anzahl	14	15	17	3	1	—	—
%		92 %		8 %			

die Differenzen steigen, wenn Titrierungen von verschiedenen Tagen miteinander verglichen werden. 10 verschiedene 0-Sera wurden mit Blutkörperchen von den obengenannten 5 Personen gemessen. Die Blutkörperchen und die Sera wurden sowohl frisch als aufbewahrt paarweise miteinander verglichen, wie aus der Tabelle IV hervorgeht. Die Ablesungen *von verschiedenen Tagen* wurden miteinander verglichen.

Vergleicht man die Zahlen aus dieser Tabelle mit denen in Tabelle III, so wird man sehen, dass die Differenzen hier deutlich grösser sind. In der Tabelle III wurde dasselbe Serum an demselben Tage untersucht, hier in Tabelle IV, Abt. 1, wurden analoge Reaktionen: dasselbe Serum contra dieselben frischen Blutkörperchen derselben Person, an zwei verschiedenen Tagen miteinander verglichen. Die Tabellen sprechen dafür, dass die Ursache der Verschiedenheiten darin liegt, dass es fast unmöglich ist, die Ablesungen der Grenzwerte an verschiedenen Tagen ganz identisch anzuführen. Die Unterschiede in Tabelle IV, Abt. 2, 3 und 4, können teils eben auf demselben Umstand, teils wiederum darauf beruhen, dass sich die Empfindlichkeit der Blutkörperchen schnell verändert hat. Auf jeden Fall sind die Differenzen in der Tabelle II ungefähr von derselben Grösse wie die in Tabelle IV: 1, wo die Reaktionen desselben Serums mit gleichempfindlichen Blutkörperchen an verschiedenen Tagen abgelesen wurden.

Um die Sache noch weiter zu beleuchten, haben wir die folgende Tabelle V zusammengestellt. In dieser sind die Resultate der Titrierungen von 160 *verschiedenen* 0-Sera und ganz frisch entnommenen Blutkörperchen derselben Empfindlichkeit miteinander verglichen.

Die Differenzen zwischen den einzelnen Reaktionen wurden weiter so berechnet, dass die erste der zweiten desselben Tages, die zweite der dritten, die dritte der vierten, usw., als Paar einander gegenübergestellt wurden. Zuletzt wurde die Differenz zwischen der letzten und der ersten Reaktion desselben Tages ermittelt. Die

TABELLE V

DIE 20 ZUERST ABGELESENEN TITERWERTE VON 8 VERSCHIEDENEN TAGEN WURDEN ZUSAMMENGESTELLT, ALSO INSGESAMT 160 VERSCHIEDENE SERA MIT FRISCHEN A_1- UND B-BLUTKÖRPERCHEN UNTERSUCHT. DIE VERTEILUNG DER RESULTATE NACH DER STÄRKE DER REAKTIONEN.

	Die Verdünnungen									
	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	Summe	Mittelwert
					O Anti-A_1					
Anzahl	—	—	1	23	52	50	26	8	160	33.18
					O Anti-B					
Anzahl	—	1	23	40	57	29	10	—	160	18.09

TABELLE VI

DIE DIFFERENZEN DER ABLESUNGEN DESSELBEN TAGES. 160 SERA.

O Anti-A_1

Differenzen in Stufen	0	½	1	1½	2	2½	3	3½	
Anzahl	47	9	67	7	23	—	6	—	= 160 »Paare»
%.....	76.9 %			23.1 %					

O Anti-B

Differenzen in Stufen	0	½	1	1½	2	2½	3	3½	4	
Anzahl	42	9	55	6	32	2	11	1	2	= 160 »Paare»
%.....	66.25 %			33.75 %						

Anzahl der Differenzen für jeden Tag betrug somit 20. Am zweiten Tage wurden die Differenzen ähnlich berechnet, so auch am dritten Tage, usw. Man erhält somit 160 Differenzen (Tabellen VI), die durchgehends nur an demselben Tage gefundenen Reaktionsdifferenzen entsprechen. Anderseits wurden die Differenzen auch so berechnet, dass die erste des ersten Tages mit der ersten am folgenden Tage, diese wieder mit der ersten des dritten Tages, usw., die zweite Reaktionsstärke des ersten Tages mit der zweiten des zweiten Tages, diese mit der zweiten des dritten Tages usw. die Reihen hindurch und die letzte des letzten Tages schliesslich mit der ersten des ersten Tages verglichen wurde. Man erhält also auch hier 160 Reaktionsdifferenzen, die alle an verschiedenen Tagen ausgeführten Ablesungen entsprechen. Siehe die Tabelle VII. Die 160 Sera waren dieselben wie in Tabellen V und VI. Die Blutkörperchen waren frisch entnommen und gleich empfindlich.

In den Tabellen VI und VII sind die 160 Reaktionen dieselben, aber die Differenzen der »Paare» verschiedenartig berechnet. In der

TABELLE VII

DIE DIFFERENZEN DERSELBEN ABLESUNGEN VON VERSCHIEDENEN TAGEN MITEINANDER VERGLICHEN. DIESELBEN 160 REAKTIONEN WIE IN TABELLE VI.

0 Anti-A_1

Differenzen in Stufen	0	½	1	1½	2	2½	3	3½	4	
Anzahl	36	5	57	9	38	4	7	2	2	= 160 »Paare«
%.....	61.25 %			38.75 %						

0 Anti-B

Differenzen in Stufen	0	½	1	1½	2	2½	3	3½	4	
Anzahl	30	7	52	8	37	5	18	—	3	= 160 »Paare«
%.....	55.6 %			44.4 %						

Tabelle VI sind die Reaktionen nur desselben Tages miteinander verglichen, in der Tabelle VII wieder stets eine bestimmte Reaktion des einen Tages mit den entsprechenden Reaktionen der folgenden Tage verglichen. Es ergibt sich eine deutliche Differenz, was dafür spricht, dass die Ablesungen der verschiedenen Tage mehr voneinander abweichen als die desselben Tages.

Wir möchten aus allen diesen Versuchen schliessen, dass es trotz Verwendung frisch entnommener Blutkörperchen kaum möglich ist, an verschiedenen Tagen ganz identische Ablesungen zu erhalten. Nur durch Verwendung einer grossen Anzahl von Serien können die technischen, methodischen und auch die Ablesungsfehler ausgeschaltet werden.

Werden die Reaktionen mit ein paar Tage alten, lege artis aufbewahrten Blutkörperchen ausgeführt, so werden sie etwas schwächer, bleiben aber unter sich vergleichbar, wie aus dem folgenden hervorgeht. In der Tabelle V wurden die 160 Reaktionen ihrer Stärke nach in Gruppen geordnet. Stellt man alle mit frisch entnommenen Blutkörperchen ausgeführten Reaktionen derselben Tage zusammen, so ergibt sich folgende Verteilung (Tabelle VIII). Die Anzahl der Reaktionen war an jedem Tage viel grösser als die 20, die in den Tabellen VI und VII wiedergegeben waren.

Wie man aus der Tabelle ersieht, war der Mittelwert mit frischen Blutkörperchen für 0 Anti-A_1 38.10 und für 0 Anti-B 20.36. Die Mittelwerte, die wir mit ein paar Tage alten Blutkörperchen erhalten haben, waren beziehungsweise 30.7 für 0 Anti-A_1 und 15.6 für 0 Anti-B. Die frischen Blutkörperchen haben etwas kräftiger reagiert. Das Verhältnis $\frac{0 \text{ Anti-}A_1}{0 \text{ Anti-B}}$ war trotzdem in beiden Fällen

TABELLE VIII

O Anti-A_1, die Blutkörperchen frisch entnommen.

Die Verdünnungen	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	Summe	Mittelwert der Titer
Anzahl	1	—	11	64	198	199	110	31	6	2	622.	38.10
%.....	0.1 %	—	1.8 %	10.3 %	31.8 %	32.0 %	17.7 %	5.0 %	1.0 %	0.3 %		

O Anti-A_1, die Blutkörperchen ein paar Tage lang aufbewahrt.

Die Verdünnungen	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	Summe	Mittelwert der Titer
Anzahl	2	—	14	54	119	83	56	9	3	—	340	30.7
%.....	0.6 %	—	4.1 %	15.9 %	35.0 %	24.4 %	16.5 %	2.6 %	0.9 %	—		

O Anti-B, die Blutkörperchen frisch entnommen.

Die Verdünnungen	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	Summe	Mittelwert der Titer
Anzahl	6	11	73	159	202	113	52	5	1	622	20.36
%.....	1.0 %	1.8 %	11.7 %	25.6 %	32.5 %	18.2 %	8.3 %	0.8 %	0.1 %		

O Anti-B, die Blutkörperchen ein paar Tage lang aufbewahrt.

Die Verdünnungen	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	Summe	Mittelwert der Titer
Anzahl	4	10	76	96	91	44	17	2	—	340	15.6
%.....	1.2 %	2.9 %	22.4 %	28.2 %	26.8 %	12.9 %	5.0 %	0.6 %	—		

ungefähr dasselbe, nämlich 1.87 und 1.97. Die Variationen der Mittelwerte, die mit frischen und ein paar Tage alten lege artis aufbewahrten Blutkörperchen erhalten werden, entsprechen nur einem Bruchteil einer Verdünnungsstufe. Die Fehler des Verhältnisses $\frac{0 \text{ Anti-A}_1}{0 \text{ Anti-B}}$ sind noch kleiner.

Zusammenfassung: An die grosse Schwierigkeit, die Ablesungen der Titrierungen konstant von Tag zu Tag zu machen wird hingewiesen. Diese Ablesungsfehler und die Bedeutung der frisch entnommenen Blutkörperchen werden eingehend analysiert.

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AUS DEM SEROBAKTERIOLOGISCHEN INSTITUT DER UNIVERSITÄT HELSINKI

ZUR KENNTNIS DER TITERWERTE BEI DEN ISOAGGLUTINATIONSREAKTIONEN UND DEREN VERWENDUNG

II. ÜBER DIE ANTHROPOLOGISCHE KONSTANZ DER TITERWERTE EINES VOLKES

Von

OSV. RENKONEN (STRENG) und ESKO SUOMALAINEN

In Tabelle I ist das dieser Arbeit zugrundeliegende Material zusammengestellt. Die untersuchten Personen waren junge Blutspender im Alter von etwa 17 bis 40 Jahren, die meisten zwischen 20 und 30 Jahren [vgl. Renkonen (Streng) (1) sowie Renkonen (Streng) und Suomalainen (2)].

TABELLE I

Die Titerwerte	2/1	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	Summe
O Anti-A_1													
Anzahl	—	3	19	220	816	2732	1794	1194	267	54	2	—	7101
%	—	0.04	0.3	3.1	11.5	38.5	25.2	16.8	3.8	0.8	0.02	—	
B Anti-A_1													
Anzahl	—	—	6	46	123	417	252	159	44	—	—	—	1047
%	—	—	0.6	4.4	11.7	39.8	24.1	15.2	4.2	—	—	—	
Anti-A_1 insgesamt													
Anzahl	—	3	25	266	939	3149	2046	1353	311	54	2	—	**8148**
%	—	0.03	0.3	3.3	11.5	38.7	25.1	16.6	3.8	0.7	0.02		
O Anti-B													
Anzahl	27	47	129	1023	1772	2457	950	358	60	5	—	—	6828
%	0.4	0.7	1.9	15.0	25.9	36.0	13.9	5.2	0.9	0.1	—	—	
A Anti-B													
Anzahl	—	—	29	307	602	1038	336	140	20	2	—	—	2474
%	—	—	1.2	12.4	24.3	41.9	13.6	5.7	0.8	0.1	—	—	
Anti-B insgesamt													
Anzahl	27	47	150	1330	2374	3495	1286	498	80	7	—	—	**9302**
%	0.3	0.5	1.7	14.3	25.5	37.6	13.8	5.3	0.9	0.1	—	—	

Wie man sieht, sind die Reaktionen für Anti-A_1 etwas stärker als für Anti-B. Der Mittelwert für O Anti-A_1 ist 32.95 und der für O Anti-B 17.6. Berücksichtigt man die Werte der einzelnen Blutkörperchenspender, variieren die Zahlen nicht viel, wie es Tabelle II zeigt.

Die mit den A_1-Blutkörperchen gemessenen Mittelwerte erscheinen unter sich nicht ganz identisch. Doch sind die Variationen ziemlich gering, von 27.49 bis 37.42. Dasselbe gilt für die B-Blutkörperchen unter sich. Die Mittelwerte liegen zwischen 16.59 und 18.97. (Der erstgenannte Mittelwert betrifft Messungen mit männlichen A_1-Blutkörperchen, die anderen beziehen sich auf weibliche Blutkörperchen.) Die mit A_1- und B-Blutkörperchen gemessenen Reaktionen differieren mehr voneinander. Die Reaktionen mit A_1-Blut sind beinahe doppelt so stark wie die mit B-Blut. Bei den einzelnen Individuen sind die Variationen der Serumstärke sehr gross.

Von den Titerwerten O Anti-A_1 (zwei A_1-Personen: Frl. K-a und Frl. H-a) wurden die Mittelwerte in Gruppen von 100 aufeinanderfolgenden Titrierungen berechnet. Die gewonnenen Resultate zeigen, dass auch die Mittelwerte der einzelnen Serien von 100 Observationen ziemlich stark variieren können, im Falle Frl. K-a von 20.36 bis 60.88, im Falle Frl. H-a von 21.08 bis 44.44. Dies zeigt, wie irreführend eine Serie von nur 100 Personen sein kann. Der Mittelwert in der ganzen Gruppe I (Frl. K-a; insgesamt 1,800 Mesungen) ist 35.13[1] und in der Gruppe II (Frl. H-a; insgesamt 2,300 Messungen) 32.03[1]. Als Unterschied der Mittelwerte ergibt sich 3.1. Der nach der Formel $M_1 = \pm \sqrt{\frac{\Sigma\,(v - v_0)^2}{r - 1}}$ berechnete Mittelfehler der Mittelwerte der einzelnen Serien (je 100 Titrierungen) in der Gruppe I ist $\pm$ 9.95 und in der Gruppe II $\pm$ 5.76. Der Mittelfehler des Mittelwertes der ganzen Gruppe I, nach der Formel $M_1 = \pm \sqrt{\frac{\Sigma\,(v - v_0)^2}{r\,(r - 1)}}$ berechnet, ist $\pm$ 2.35 und derjenige der Gruppe II $\pm$ 1.20. Der Mittelfehler der Differenz zwischen den beiden ganzen Seriengruppen, nach der Formel $M_\Delta = \pm \sqrt{M_1^2 + M_2^2}$ berechnet, ist $\pm$ 2.64, während die Differenz der Mittelwerte 3.10 war. Dies zeigt, dass grössere Unterschiede zwischen den Blutkörperchen der zwei genannten A_1-Personen nicht vorhanden sind. Vergleicht man die Reaktionen der Blutkörperchen der zwei oben-

[1] Die entsprechenden Mittelwerte in der Tabelle II gründen sich auf eine etwas verschiedene Zahl von Titrierungen und sind darum ein wenig abweichend.

TABELLE II

TITERWERTE JE NACHDEM, WELCHE PERSONEN DIE BLUTKÖRPERCHEN FÜR DIESE TITRIERUNGEN GELIEFERT HATTEN.

O Anti-A_1

Blutkörperchen von	Die Verdünnungen												Summe	Mittelwerte der Titer
	2/1	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024		
Frl. H-a[1]														
Anzahl	—	1	5	95	305	963	652	410	80	13	—	—	2524	31.26
%	—	0.03	0.2	3.8	12.1	38.2	25.8	16.2	3.2	0.5	—	—		
Frl. N-i														
Anzahl	—	1	2	32	120	506	320	290	67	16	—	—	1354	37.42
%	—	0.1	0.1	2.4	8.9	37.4	23.6	21.4	4.9	1.2	—	—		
Frl. K-a														
Anzahl	—	—	7	38	167	671	473	295	68	23	1	—	1743	35.20
%	—	—	0.4	2.2	9.6	38.5	27.1	16.9	3.9	1.3	0.1	—		
Herr U-a														
Anzahl	—	1	1	26	128	267	169	77	17	2	1	—	689	27.49
%	—	0.1	0.1	3.8	18.6	38.8	24.5	11.2	2.5	0.3	0.1	—		
N.N.[2] Anzahl	—	—	4	29	96	325	180	122	34	—	—	—	790	30.39
%	—	—	0.5	3.7	12.2	41.1	22.8	15.4	4.3	—	—	—		
Insgesamt	—	3	19	220	816	2732	1794	1194	267	54	2	—	7101	32.95
%	—	0.04	0.3	3.1	11.5	38.5	25.2	16.8	3.8	0.8	0.03	—		

[1] Frl. H-a = H + K-n bei Renkonen (1). Hier sind H und K-n zusammengenommen, weil die Person dieselbe war (H-a = Vorname, K-n = Zuname).
[2] In den Protokollen nicht angegeben.

O Anti-B

Blutkörperchen von	Die Verdünnungen												Summe	Mittelwerte der Titer
	2/1	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1204		
Frau K-o														
Anzahl	10	21	41	415	770	1188	425	181	27	2	—	—	3080	18.21
%	0.3	0.7	1.3	13.5	25.0	38.6	13.8	5.9	0.9	0.06	—	—		
Frl. Sch-n														
Anzahl	17	26	77	512	804	984	394	137	22	3	—	—	2976	16.59
%	0.6	0.9	2.6	17.2	27.0	33.1	13.2	4.6	0.7	0.1	—	—		
Frl. S-i														
Anzahl	—	—	—	1	5	7	—	—	—	—	—	—	13	—
Frl. S-d														
Anzahl	—	—	—	—	—	1	5	2	—	—	—	—	8	—
N.N.[3] Anzahl	—	—	11	95	193	277	126	38	11	—	—	—	751	18.97
%	—	—	1.5	12.6	25.7	36.9	16.8	5.0	1.5	—	—	—		
Insgesamt	27	47	129	1023	1772	2457	950	358	60	5	—	—	6828	17.60
%	0.4	0.7	1.9	15.0	25.9	36.0	13.9	5.2	0.9	0.1	—	—		

[3] In den Protokollen nicht angegeben.

genannten Personen in Serien von 1,000, so ist die Differenz der Mittelwerte ungefähr dieselbe, 3.6, aber der Mittelfehler der Differenz kleiner, $\pm$ 0.7, was somit vielleicht doch auf das Vorhandensein eines ganz kleinen realen Unterschiedes hindeutet.

Die Differenz zwischen Frau K-o und Frl. Sch-n in bezug auf O Anti-B zeigt dasselbe. Teilt man das Material in Gruppen von 100 Titrierungen ein, so findet man als Differenz der Mittelwerte der beiden ganzen Seriengruppen 18.21—16.59 = 1.62. Der Mittelfehler der Differenz der Mittelwerte der zwei Seriengruppen ist $\pm$ 1.19. Auch hier war es nicht möglich, einen realen Unterschied zwischen den zwei B-Blutkörperarten herauszufinden. Dessenungeachtet haben sich auch hier deutliche Unterschiede zwischen den Mittelwerten der zwei Blutkörperchen in den einzelnen Serien von 100 Reaktionen ergeben (im Falle Frl. Sch-n von 9.75 bis 25.75, im Falle Frau K-o von 9.40 bis 32.06). Dasselbe ist auch in betreff der anderen Blutspender zu sehen. Dies bedeutet, dass sowohl die Blutkörperchen der von uns für die vergleichenden Reaktionen ausgewählten A_1-Personen unter sich und die der B-Personen unter sich ungefähr dieselbe Empfindlichkeit hatten. Die Differenz zwischen Anti-A_1 und Anti-B ist dagegen real. Die Differenz der Mittelwerte war 32.95—17.60 = 15.35. Diese Differenz ist so gross, dass sie deutlich auf einen realen Unterschied zwischen O Anti-A_1 und O Anti-B hindeutet.

Reiht man unabhängig von den Blutkörperchenspendern die Reaktionen in Gruppen von 100 aneinander, so findet man, dass die Zahlen betreffs O Anti-A_1 von 21.8 bis 60.88 und betreffs O Anti-B von 12.18 bis 27.92 variieren. Die Differenzen der Mittelwerte können (infolge verschiedener Ablesung usw.) um mehr als das Doppelte variieren.

Von dem Gedanken ausgehend, dass die Fehler der Ablesung kleiner werden, wenn man die *Verhältnisse* $\frac{\text{O Anti-A}_1}{\text{O Anti-B}}$ in Serien von 100 Anti-A_1-Werten und 100 Anti-B-Werten ausrechnet und miteinander vergleicht, haben wir unser Material auch auf diese Art untersucht. Vom Ableser an demselben Tage bestimmte Grenzwerte für O Anti-A_1 und O Anti-B wurden somit miteinander verglichen. In 20 solchen Serien von 100 Titrierungen (insgesamt also 2,000 Titrierungen) wurden die Mittelwerte für Anti-A_1 und Anti-B berechnet und danach das Verhältnis der Mittelwerte $\frac{\text{O Anti-A}_1}{\text{O Anti-B}}$

in jeder Serie bestimmt. Die so erhaltenen Werte variieren von 1.38 bis 2.77. Der Mittelwert für $\frac{\text{O Anti-A}_1}{\text{O Anti-B}}$ ist 1.92 und der Mittelfehler des Mittelwertes nach der Formel $M_1 = \pm \sqrt{\frac{\Sigma\ (v-v_0)^2}{r\ (r-1)}}$ berechnet $\pm$ 0.09.

In einer Serie wurde für O Anti-A_1 ein abnorm hoher Wert (60.88) notiert. Das Verhältnis $\frac{\text{O Anti-A}_1}{\text{O Anti-B}} = 2.18$ wiech jedoch nicht viel vom Mittelwert 1.92 ab, weil auch der Wert für O Anti-B in derselben Serie vom Ableser abnorm hoch (27.92) geschätzt worden war. Dasselbe gilt auch für einige andere untersuchte Serien. Nur in drei von zwanzig Serien wichen die Verhältniszahlen mehr ab; in diesen lauteten die Werte für das Verhältnis $\frac{\text{O Anti-A}_1}{\text{O Anti-B}}$ auf 2.77, 2.72 und 2.66. Wir finden es somit nicht hinreichend, nur die Werte für O Anti-A_1 und O Anti-B festzustellen, sondern auch vorteilhaft immer das Verhältnis $\frac{\text{O Anti-A}_1}{\text{O Anti-B}}$ in mehreren Serien von 100 auszurechnen. *Hierdurch werden die wichtigsten Fehler, nämlich die der Ablesungsart einigermassen ausgeschaltet.*

Gruppiert man unsere Zahlen in Serien von nur 50 Individuen, so findet man den Mittelwert für die ersten 10 Gruppen von 50 Individuen nur etwas niedriger, 1.64, und den Mittelfehler der Mittelwerte etwas grösser, $\pm$ 0.12. Wir haben ungefähr denselben Mittelwert für $\frac{\text{O Anti-A}_1}{\text{O Anti-B}}$ in den verschiedensten Kombinationen in Finnland gefunden. In Tabelle VIII (2) mit ganz frischen Blutkörperchen wurde $\frac{\text{O Anti-A}_1}{\text{O Anti-B}} = \frac{38.10}{20.16} = 1.87$ gefunden. Mit meistens ein paar Tage alten Blutkörperchen ergab sich, nach derselben Tabelle, das Verhältnis $\frac{30.7}{15.6} = 1.97$. In der Tabelle V (2) war $\frac{\text{O Anti-A}_1}{\text{O Anti-B}} = \frac{33.18}{18.09} = 1.84$. In der Tabelle II (das Gesamtmaterial, S. 273) war das Verhältnis $\frac{32.95}{17.60} = 1.87$. Wir glauben daher der Ansicht sein zu können, dass unsere Titrierungen die Verhältnisse in Finnland ziemlich richtig wiedergeben. Bemerkenswert ist, dass unsere Verhältniszahlen für $\frac{\text{O Anti-A}_1}{\text{O Anti-B}}$ ganz andere als diejenigen in Dänemark sind.

Das Verhältnis $\frac{\text{B Anti-A}_1}{\text{A Anti-B}}$ haben wir in Finnland, ganz ähnlich wie Thomsen und Kettel (3) in Dänemark konstatiert haben, kleiner als das Verhältnis $\frac{\text{O Anti-A}_1}{\text{O Anti-B}}$, nämlich 1.65, gefunden. Auch diese Verhältniszahlen aus Dänemark sind aber andere als diejenigen in Finnland. Als Ergänzung zur Tabelle II werden unsere Resultate mit B Anti-A_1 und A Anti-B in derselben Art wie dort wiedergegeben (Tabelle III).

TABELLE III

B Anti-A_1

Blutkörperchen von	Die Verdünnungen										Summe	Mittelwerte der Titer
	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512		
Frl. H-a Anzahl	—	1	15	46	138	97	65	14	—	—	379	30.98
%	—	0.3	4.0	12.1	37.2	25.6	17.1	3.7	—	—		
Frl. N-i Anzahl	—	5	21	51	183	99	67	18	—	—	441	29.30
%	—	1.1	4.8	11.6	40.8	22.4	15.2	4.1	—	—		
Frl. K-a Anzahl	—	—	1	11	36	16	8	5	—	—	77	30.29
%	—	—	1.3	14.2	46.7	20.8	10.4	6.5	—	—		
Herr U-a Anzahl	—	—	3	5	10	11	4	2	—	—	35	30.74
N.N.[1] Anzahl	—	—	6	10	50	29	15	5	—	—	115	29.96
Insgesamt	—	6	46	123	417	252	159	44	—	—	1047	30.30
%	—	0.6	4.4	11.7	39.8	24.1	15.2	4.2	—	—		

A Anti-B

Blutkörperchen von	Die Verdünnungen										Summe	Mittelwerte der Titer
	2/1	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	
Frau K-o Anzahl	—	—	14	117	354	679	214	88	12	1	—	1539	18.65
%	—	—	0.9	11.5	23.0	44.1	13.9	5.7	0.8	0.1	—		
Frl. Sch-n Anzahl	—	—	13	97	191	240	80	32	4	1	—	658	16.96
%	—	—	2.0	14.7	29.0	36.5	12.1	4.9	0.8	0.1	—		
N.N.[1] Anzahl	—	—	2	33	57	119	42	20	4	—	—	277	20.33
%	—	—	0.7	11.9	20.6	43.0	15.2	7.2	1.4	—	—		
Insgesamt	—	—	29	307	602	1038	336	140	20	2	—	2472	18.39
%	—	—	1.2	12.4	24.3	41.9	13.6	5.7	0.8	0.1	—		

[1] In den Protokollen nicht angegeben.

Das Verhältnis $\frac{\text{B Anti-A}}{\text{A Anti-B}}$ beträgt $\frac{30.30}{18.39} = 1.64$ und ist somit etwas kleiner als $\frac{\text{O Anti-A}}{\text{O Anti-B}}$ (= 1.92). Der Unterschied ist klein, steht aber im Einklang damit was in Dänemark gefunden (3) worden ist. Auch Schiff (4) sowie Schiff und Mendlowicz (5) haben in Berlin etwas Ähnliches, doch mehr ausgeprägt gefunden. $\frac{\text{O Anti-A}}{\text{O Anti-B}}$ war dort 1.6. $\frac{\text{B Anti-A}}{\text{A Anti-B}}$ war ungefähr 1. Das Alter war nicht angegeben und die Anzahl der untersuchten B-Sera nur 34, die der A-Sera nur 68.

Aus den von Thomsen und Kettel (3) in den verschiedenen Altersgruppen gefundenen Mittelwerten haben wir die Werte für $\frac{\text{O Anti-A}}{\text{O Anti-B}}$ in Dänemark bestimmt (Tabelle IV).

TABELLE IV

$\frac{\text{O ANTI-A}}{\text{O ANTI-B}}$, BERECHNET VON UNS NACH DÄNISCHEM MATERIAL BEI THOMSEN UND KETTEL (3).

1—2 J.	2—5 J.	5—10 J.	10—20 J.	20—30 J.	30—40 J.	40—50 J.	50—60 J.	60—70 J.	70—80 J.	80—90 J.	90—100 J.
130	287	386	332	291	246	179	174	118	149	81	38
69	124	162	139	105	76	53	46	37	42	28	38
1.88	2.31	2.38	2.39	2.77	3.24	3.38	3.78	3.19	3.55	2.89	1.0

Die grösse der O-Gruppen ist in der Arbeit von Thomsen und Kettel nicht angegeben. Doch kann man wohl annehmen, dass die Anzahl der O-Individuen in jeder Hundert-Gruppe etwa 60 Personen umfasst, welches dem Frequenzprozent der O-Individuen in Dänemark entspricht. Der Mittelwert nimmt sowohl betreffs O Anti-A als betreffs O Anti-B bei den Erwachsenen mit dem Alter ab. Das Verhältnis $\frac{\text{O Anti-A}}{\text{O Anti-B}}$ bleibt in diesen Altersgruppen ziemlich unverändert. Der Mittelwert ist 3.27. Als Mittelfehler ergibt sich für das dänische Material nach unseren Berechnungen $\pm$ 0.18. In Finnland war der Mittelwert 1.92 und der Mittelfehler $\pm$ 0.09 (S. 275). Die Differenz der Mittelwerte in Dänemark und

Finnland ist 1.35, die der Mittelfehler $\pm$ 0.20, also 6 bis 7 mal kleiner als die Differenz der Mittelwerte. Es scheint somit in dieser Hinsicht zwischen Dänemark und Finnland ein realer Unterschied zu bestehen. Man kann zwar einwenden, dass die miteinander verglichenen Serien nicht gleich gross waren. In Finnland befanden sich die Untersuchten zur Hauptsache im Alter von 20—30 Jahren, im dänischen Material sind dagegen mehrere Altersgruppen repräsentiert. Wir haben deshalb die finnischen Zahlen in kleinere Gruppen von 50 eingeteilt (vgl. S. 275). Der Mittelwert war jetzt, aus 10 Serien berechnet, 1.64 und der Mittelfehler des Mittelwertes $\pm$ 0.12. Vergleicht man nun diese Zahlen mit den dänischen, so kommt man zu einem ziemlich ähnlichen Resultat wie oben. Die Differenz der Mittelwerte beträgt etwa das 8fache des Mittelfehlers der Differenz und ist also auf jeden Fall eine reale. In der Gruppe der 20—30jährigen ist der dänische Mittelwert für $\frac{\text{O Anti-A}}{\text{O Anti-B}} = 2.77$. Die Differenz zwischen Dänemark und Finnland ist in diesem Falle nur 0.82, also mehr als 4 mal grösser als die Differenz der Mittelwerte.

Wir haben, wie gesagt, aus den Zahlen von Thomsen und Kettel (3) ausgerechnet, dass das Verhältnis $\frac{\text{O Anti-A}}{\text{O Anti-B}}$ der Erwachsenen in Dänemark $\frac{332}{112}$ oder 3.0 ist. In Dänemark scheint mithin gleichfalls ebenso wie in Finnland eine merkwürdige und unbegreifliche »Korrelation» zwischen dem Verhältnis $\frac{\text{Frequenz von A}}{\text{Frequenz von B}} = 3.2$ und demjenigen der Titer von $\frac{\text{O Anti-A}}{\text{O Anti-B}} = 3.0$ zu bestehen. Die entsprechenden »Korrelationszahlen» aus Finnland waren 1.87 und 1.9.

Unsere Zahlen des gesamten Materials hatten keine wesentlichen Unterschiede betreffs der Titerwerte zwischen den Individuen mit finnischen und mit schwedischen Namen gezeigt. Unsere Titrierungen erstreckten sich aber über eine Zeit von mehreren Jahren, von 1941 bis 1944. Die an verschiedenen Tagen erhaltenen Titerwerte sind nicht ohne weiteres miteinander vergleichbar. Es ist ja weiter a priori klar, dass der Name keineswegs ein sicheres Kriterium der Nationalität ist. Ebensowenig ist es bei uns die

Sprache. Es gibt überhaupt kaum ein Kriterium in unserem gemischten Volke, welches eine sichere Antwort auf die Frage, ob »Schwede» oder »Finne», geben könnte. Allein, bestünde bei uns nun tatsächlich ein Unterschied zwischen den »Nationalitäten», der seinen Ausdruck u.a. in einer verschiedenen Titerstärke nähme, so könnte zu erwarten sein, dass uns ein diesbezüglicher Vergleich von Individuen mit finnischen und schwedischen Namen — d.h. natürlich unter der Vorbedingung einer genauen Komparabilität der Titerwerte — einen Hinweis auf jenen Unterschied liefern würde. Um die erwähnte Vorbedingung zu erfüllen, haben wir unseren Vergleich in folgender Art durchgeführt.

Die Titerstärke von 500 Individuen mit schwedischen Namen wurde mit der Serumstärke von 500 Individuen mit finnischen Namen verglichen. Diejenigen mit schwedischen Namen waren täglich in der Minderzahl vorhanden. Wenn in den Serien an einem Tage 1, 2, 3 oder mehrere Individuen mit schwedischen Namen vorkamen, wurden die Titerwerte derselben ohne Auswahl mit der Serumstärke von ebenso vielen Individuen mit finnischen Namen *desselben Tages* verglichen, und zwar standen die letzteren in der Reihenfolge stets nach dem betreffenden Individuum mit schwedischem Namen. Die als Massstab benutzten Blutkörperchen waren immer dieselben, denselben A_1- und B-Individuen entnommen. Dies dürfte unserer Auffassung nach eine genügende Vergleichbarkeit der beiden untersuchten Gruppen gewähren. Siehe die Tabelle V.

Das in der Tabelle V behandelte Material wurde weiter in 5 einander entsprechende Untergruppen (100 in jeder Gruppe) eingeteilt. In allen diesen wurde der Mittelwert für das Verhältnis $\frac{\text{O Anti-A}_1}{\text{O Anti-B}}$ berechnet. Die ermittelten fünf Mittelwerte betrugen bei den Individuen mit finnischen Namen 1.83, 1.53, 2.22, 1.81 und 2.07 und bei den Individuen mit schwedischen Namen 2.06, 1.38, 1.84, 1.98 und 2.46. Berechnet man nun auf Grund dieser Werte den Mittelwert für die 500 Blutspender mit finnischen Namen, so ergibt sich der Wert 1.89; die entsprechende Zahl bei den »schwedischen» Blutspendern ist 1.94. Der Mittelfehler des Mittelwertes beläuft sich im ersteren Fall auf $\pm$ 0.12 und im letzteren auf $\pm$ 0.17. Die Differenz dieser beiden Mittelwerte ist 0.05 und der Mittelfehler dieser Differenz $\pm$ 0.21.

Weil der Mittelfehler der Differenz $\pm$ 0.21 und die Differenz der

TABELLE V

DIE TITERWERTE VON INDIVIDUEN MIT FINNISCHEN UND MIT SCHWEDISCHEN NAMEN.

Die Anzahl	Die Serumverdünnungen									Die Mittelwerte der Titer
	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	
					O Anti-A_1					
					Individuen mit finnischen Namen					
500	—	—	7	43	119	149	78	20	4	33.80
in %	—	—	1.4	8.6	39.8	29.8	15.6	4.0	0.8	
					Individuen mit schwedischen Namen					
500	1	1	20	57	180	132	85	21	3	33.08
in %	0.2	0.2	4.0	11.4	36.0	26.4	17.0	4.2	0.6	
					O Anti-B					
					Individuen mit finnischen Namen					
500	7	5	81	108	198	62	36	3	—	18.09
in %	1.4	1.0	16.2	21.6	39.6	12.4	7.2	0.6	—	
					Individuen mit schwedischen Namen					
500	11	11	66	131	176	80	19	6	1	17.55
in %	2.2	2.2	13.2	26.2	35.2	16.2	3.8	1.2	—	

Die Individuen mit finnischen Namen: $\frac{\text{O Anti-A}_1}{\text{O Anti-B}} = \frac{33.80}{18.09} = 1.87$

Die Individuen mit schwedischen Namen: $\frac{\text{O Anti-A}_1}{\text{O Anti-B}} = \frac{33.08}{17.55} = 1.88$

Mittelwerte in den beiden Serien nur 0.05 war, so ist es offenbar, dass ein realer Unterschied betreffs des Verhältnisses $\frac{\text{O Anti-A}_1}{\text{O Anti-B}}$ zwischen den Individuen mit finnischen und schwedischen Namen in Finnland nicht existiert. Dasselbe kann auch betreffs der Werte für O Anti-A_1 und O Anti-B im einzelnen gesagt werden. Damit will aber keineswegs gesagt sein, dass sich im Lande nicht evtl. lokale Unterschiede finden könnten. Solche Unterschiede wird man wahrscheinlich nicht nur zwischen den »Finnen» und »Schweden», sondern auch zwischen den »Finnen» unter sich und den »Schweden» unter sich in verschiedenen Gegenden des Landes finden. Unser Material gibt in dieser Hinsicht keine Auskunft, weil in unseren Protokollen der Geburtsort nicht angegeben war.

Während des Krieges 1941—1944 mass der Assistent des Serobakteriologischen Instituts, Dr. T. Savolainen (6), in einer kleinen

Serie finnische und deutsche Sera mit finnischen Blutkörperchen und fand dabei Unterschiede. Die deutschen Sera reagierten schwächer. Es sind wohl grössere Serien nötig, um dies endgültig zu beweisen (vgl. S. 272—274, wo auf die Notwendigkeit grosser Serien hingewiesen wird). Das Alter ist bei Savolainen nicht angegeben; doch spielt auch das Alter in so weit eine Rolle bei diesartigen Versuchen, als nur Individuen desselben Alters unter sich verglichen werden können.

Zum Schluss haben wir noch ganz nebenbei untersucht, ob die Titerwerte der Männer von denjenigen der Frauen abweichen. Die Werte der Männer, deren Anzahl klein war, wurden zuerst ausgesucht und danach die entsprechenden Werte der in der Reihe nächstfolgenden Frauen. Die Ergebnisse der Zusammenstellung erhellen aus Tabelle VI.

TABELLE VI

DIE TITERWERTE DER MÄNNER UND FRAUEN.

	Die Anzahl	Die Serumverdünnungen								Die Mittelwerte der Titer
		1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	
		O Anti-A_1								
Die Frauen	250	—	—	4	25	99	61	52	9	32.9
Die Männer	250	—	—	5	33	101	71	33	7	28.8
		O Anti-B								
Die Frauen	250	5	1	38	69	91	29	14	3	17.5
Die Männer	250	5	9	46	78	78	24	9	1	14.2
		B Anti-A_1								
Die Frauen	69	—	—	3	4	36	14	9	3	29.5
Die Männer	69	—	—	1	13	30	15	6	4	22.9
		A_1 Anti-B								
Die Frauen	215	—	—	19	52	99	30	14	1	18.9
Die Männer	215	—	1	28	47	95	34	9	1	17.7

Bei den Männern war das Verhältnis $\frac{\text{O Anti-A}_1}{\text{O Anti-B}} = 2.0$, bei den Frauen 1.9, also beinahe dasselbe.

Man sieht, dass die Unterschiede nicht gross sind, in sämtlichen Gruppen liegen jedoch die Mittelwerte der Frauen etwas über denjenigen der Männer. Wir möchten aus diesen Zahlen keinerlei Schlüsse ziehen, insbesondere weil das genaue Alter der Spender

uns nicht bekannt war, aber wir finden, dass die Frage nach eventuellen Unterschieden näher untersucht zu werden verdiente. Auffallend ist jedenfalls der Umstand, dass die Mittelwerte für Anti-A_1 und Anti-B in allen Kombinationen bei den Frauen grösser als bei den Männern waren. Schiff und Mendlowicz (5) haben keine nennenswerten Unterschiede zwischen Männern und Frauen gefunden. Die Anzahl der untersuchten Individuen war jedoch gering.

SCHLUSSFOLGERUNGEN

Unter der Voraussetzung einer genügend grossen Anzahl Sera von Personen im Alter von etwa 20 bis 40 Jahren und mittelempfindlicher Blutkörperchen, einer gleichmässigen Technik und Ablesung, scheinen ziemlich konstante Mittelwerte der Titer erreichbar zu sein. Für vergleichende Versuche am besten verwendbar sind die Verhältniszahlen $\frac{\text{O Anti-A}_1}{\text{O Anti-B}}$. Das Verhältnis der Titerwerte $\frac{\text{O Anti-A}_1}{\text{O Anti-B}}$ scheint im Alter von 20—40 Jahren in Finnland ziemlich konstant bei etwa 1.8—1.9 zu liegen. O Anti-A_1 ist somit etwa doppelt so stark wie O Anti-B. B Anti-A_1 scheint nur etwas schwächer als O Anti-A_1 zu sein. A_1 Anti-B entspricht ungefähr O Anti-B. Die Titerwerte für O Anti-A_1 und B Anti-A_1 folgen einander, ebenso die Werte für O Anti-B und A_1 Anti-B.

In Dänemark ergeben sich anscheinend andere Verhältniszahlen als in Finnland (Thomsen und Kettel, 3). Das Verhältnis $\frac{\text{O Anti-A}_1}{\text{O Anti-B}}$ ist dort 3.0 und die Stärke von O Anti-A_1 somit etwa 3 mal so gross wie die von O Anti-B. Die Mittelwerte der Titer für O Anti-A_1 und B Anti-A_1 und ebenso die Werte für O Anti-B und A_1 Anti-B scheinen in beiden Ländern den Frequenzwerten für A_1 und B zu folgen und in derselben Altersgruppe ziemlich konstant zu sein.

Zwischen Individuen mit finnischen und schwedischen Namen sind in Finnland keine Unterschiede betreffs der Mittelwerte für O Anti-A_1 und O Anti-B und deren Verhältnisse vorhanden.

Da es schon mit Hilfe verhältnismässig kleiner Blutuntersuchungsserien (einige hundert Sera) möglich ist, ziemlich konstante Werte für $\frac{\text{O Anti-A}}{\text{O Anti-B}}$ zu finden, so wäre es wohl nicht schwierig fest-

zustellen, ob sich vielleicht bei anderen Völkern ähnliche oder verschiedene Werte feststellen liessen.

Bei den Frauen scheinen die Mittelwerte der Titer für O Anti-A_1 und O Anti-B ein bisschen grösser als bei den Männern zu sein. Diese Frage müsste aber näher untersucht werden. Die kleinen Unterschiede könnten auch von kleineren Altersdifferenzen herrühren.

Die grosse Konstanz der Frequenz der verschieden starken Reaktionen wird nach unserer Meinung am besten durch die Annahme verständlich, dass die agglutinierenden Stärken von Anti-A_1 und Anti-B, also $\alpha\alpha$ und $\beta\beta$, bei der Isoagglutination grösstenteils erblich bedingt sind. α- und β- Gene brauchen jedoch nicht Allelen zu sein.

Auf die merkwürdige Korrelation $\frac{\text{Frequenz A}}{\text{Frequenz B}} = \frac{\text{Titerstärke } \alpha}{\text{Titerstärke } \beta}$ bei ein und demselben Volk wird hingewiesen.

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FROM THE WIHURI RESEARCH INSTITUTE

EFFECT OF LUMBAR SYMPATHECTOMY ON EXPERIMENTAL ARTHRITIS

By

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In a previous paper we have shown that tetraethyl ammonium bromide (TEAB) inhibits the production of formalin arthritis (2). TEAB is known to have an inhibiting effect on both sympathetic and parasympathetic impulses. To investigate the part played by the sympathetic nervous system in formalin arthritis we carried out the following series of experiments:

As experimental animals, white male rats weighing 200—300 g were used, on six of which lumbar sympathectomy was made as follows:

The rats were kept fasting for 24 hours, after which they were anaesthetized with evipan (0.025 g of evipan-Na). A median abdominal incision was made. The lumbar portion of the sympathetic trunk was approached from the left side, while the intestines were pushed to the right. The left iliolumbar vein and artery were ligated, after which the kidney was caused to move obliquely upwards and to the right. The left genitofemoral nerve was moved a little to the left, and from beneath the peritoneum the sympathetic trunk was dissected in the immediate neighbourhood of the vena cava, and a piece about 1 cm in length removed from it. It was checked histologically that this belonged to the sympathetic trunk.[1]) Catgut and silk were used to suture the abdominal wall.

[1] In this connection we wish to express our gratitude to Dr. E. Saxen, who kindly made the histological investigations.

On six control rats a similar incision was made, with the difference, however, that the sympathetic trunk was not touched. The control animals and the sympathectomized ones were kept under identical environmental conditions. They were each of them given a formalin injeetion ten days after the operation. The reaction caused by the formalin injection was followed in the way described earlier (1).

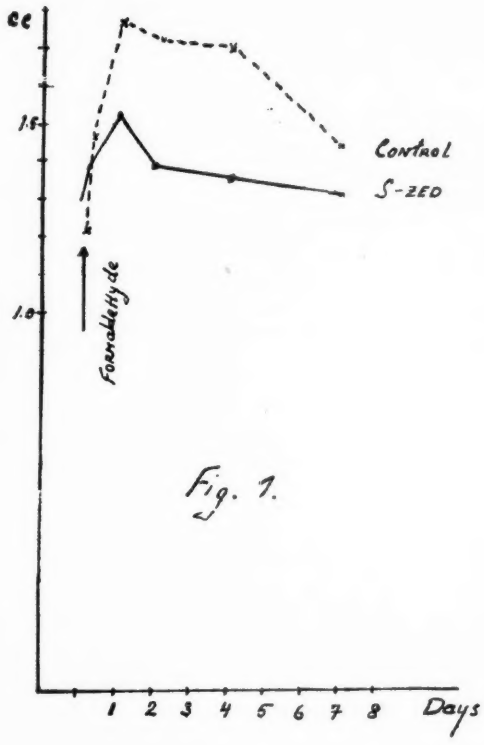

Fig. 1.

Macroscopical examination revealed that in the left hind leg of the rats the reaction was as a rule stronger than in the right, which may be due to the ligation of the iliolumbar vessels on the left side.

On the other hand, when comparing with each other the control rats and those that were sympathectomized, it was found that the formalin reaction definitely developed more slowly and weakly in the sympathectomized rats. The reaction correspondingly

disappeared considerably more rapidly in them than in the control rats. This can be observed in Fig. 1, which shows the daily mean volumes of the hind legs in both groups of experimental animals.

It is also to be seen that the reaction in the control rats is rapid and strong, reaching a maximum within 24 hours, and continuing almost as great during the next three days, then decreasing rather sharply. Eight days after the formalin injection, however, the volume of the legs (swellings) is still remarkably large compared with the original values. In the sympathectomized rats the reaction develops more slowly and weakly; however, it reaches a maximum in them also within one day, clearly decreasing already on the following day. By the eight day the swellings have disappeared and the volume of the legs is the same as before the formalin injection.

Although the number of animals used in our experimental series was rather small, the differences in the intensities of the reactions were, however, so clear in both groups of experimental animals that we may regard it as probable that lumbar sympathectomy inhibits the production of formalin arthritis in rats. In this connection it may be mentioned that sympathectomy has sometimes also been observed to be beneficial in polyarthritis in man (3, 4).

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FROM THE DEPARTMENT OF ANATOMY, UNIVERSITY OF HELSINKI

DEMONSTRATION OF ACID PHOSPHATASE IN TISSUE SECTIONS

AN IMPROVED METHOD

By

OLAVI ERÄNKÖ

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About ten years ago, Gomori published his methods for the demonstration of alkaline (1) and acid (2) phosphatases in tissue sections. Since then, numerous studies on the distribution of phosphatases in various organs and tissues have been published. Reproducible results have been obtained with the technique for alkaline phosphatase, but, as pointed out by Lison (3) in a recent review, results obtained with the acid phosphatase method vary capriciously. Papers dealing with the histochemistry of acid phosphatase are therefore few in comparison to those concerning the alkaline phosphatase.

Fixation in acetone, paraffin embedding, and incubation at 37° C for 6—48 hours are generally recommended for the acid phosphatase technique. Cytological preservation is, however, not good in specimens treated according to this procedure, and irregular phosphatase negative areas are often seen in the sections.

In a series of experiments we observed that 4 per cent formalin (one part of commercial formaldehyde solution and nine parts of distilled water) neutralized over calcium carbonate was a very suitable fixative for the present purpose and superior to all other fixatives tested. Further, the use of frozen sections instead of

paraffin embedding essentially simplified the technique and allowed the use of short immersion times in the substrate solution. The following method is recommended.

THE METHOD

(1) Fixation of small pieces in 4 per cent formalin for 2—6 hours at room temperature or for 12—24 hours at 0—3° C. (2) Cutting with a freezing microtome at 10—40 micra. (3) Washing of sections in two rinses of distilled water. (4) Immersion in the glycerophosphate solution (2) for 2--240 mins. (5) Washing in three rinses of distilled water. (6) Floating on slides prepared with glycerol albumen. (7) After drying, immersion of slides in 0.1 per cent celloidin for 5 min. (8) Hardening in 70 per cent alcohol for 5 min. (9) Washing in distilled water. (10) Immersion in a solution containing one part of fresh yellow ammonium polysulphide in nine parts of water for 1—2 min. (11) Washing in water. (12) Mounting in balsam after drying or through alcohol and xylene series. Alternatively the sections can be first treated with ammonium polysulphide and then floated on slides. On the other hand, it is possible to attache the sections on slides before the immersion in the glycerophosphate.

For comparison, fresh-frozen sections fixed for 2—5 min. in 4 per cent formalin were attached on slides and handled identically with sections cut from previously fixed tissues.

RESULTS

This method has been successfully applied to many organs of the rat. Similar tissues always reacted consistently, and no patchy negative areas were seen. The length of the treatment in the substrate solution must be tested for each organ separately. In the adrenal medulla, for instance, a definitely positive reaction may be seen in 15 mins. at room temperature, while an hour is required for the appearance of discernible darkening of the adrenal cortex. The temperature of the substrate-buffer mixture can be varied from 10° C to 50° C, room temperature being convenient for many purposes. The acid phosphatase reaction of some organs is essentially modified by a previous treatment with, *e.g.*, alcohol, with the

histochemical reaction weakening considerably. Besides, distributions of phosphatase positive materials in similar tissues sometimes differ from each other in frozen sections and in acetone-fixed paraffin sections. This may be, at least partly, due to diffusion of the enzyme and/or of lead phosphate during the long incubation period required when paraffin sections are used (cp. 4). Cytological structures are well preserved with the new method.

Sites of positive reactions were further different in some organs depending on the method of fixation. For instance, the adrenal medullary cells showed a strong cytoplasmic and no nuclear reaction in fresh-frozen sections but a definite nuclear and only a weak cytoplasmic darkening in sections from glands fixed for some hours. This probably indicates that the enzyme is prone to diffuse, not only in the course of the incubation (4) but also during the fixation. Further studies are in progress.

SUMMARY

An improved method for the histochemical demonstration of acid phosphatase is described. In this method frozen sections of the formalin-fixed specimens are immersed in the substrate solution. Results thus obtained are reproducible. Cytological preservation is good. Presumable evidence of diffusion of the enzyme during the fixation period is presented.

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FROM THE VIRUS LABORATORY OF THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY, HELSINKI UNIVERSITY, AND STATE SERUM INSTITUTE

STUDIES ON THE SEROLOGIC DIAGNOSIS OF MUMPS

By

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(Received for publication January 30, 1951)

The serologic diagnosis of mumps is not yet a common laboratory method, as indicated by the relatively few publications concerning the subject (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12). Serological methods are, however, of importance in diagnosing atypical forms of mumps virus infections, such as meningitis, orchitis, or pancreatitis (9, 12). If paired (acute and convalescent phase) sera can be collected, the methods are proven to be useful. By testing, however, only one or two specimens collected later in the convalescence, the serological diagnosis may remain obscure because of the high percentage of antibodies against mumps virus in a normal population. The object of this study was to obtain a notion of the anti-mumps-virus titers in the Finnish population in general and after clinically ascertained mumps infections. The results may be of value in evaluating the serological titers in suspected cases of mumps and give a notion of the titer changes during a period of 8 months after infection.

MATERIAL AND METHODS

The anti-mumps-virus titers in the population in general are represented by the results obtained by testing 44 sera from medical

[1] Aided by a grant from the Sigrid Juselius Foundation.
[2] With the technical assistance of Mrs. Pirkko Koivunen.

students and 9 sera from children[1] of 11 to 17 months of age. The sera in 23 clinically certain cases of mumps[2] were tested in order to follow the changes in anti-mumps-virus titer after the infection. From 1 to 4 specimens were collected from each patient. The time interval between the onset of symptoms and the collecting of specimens varied between 2 days and 8 months.

Complement Fixation Technique. Antigen. — Eggs incubated at 37.5°C for seven to eight days were infected by the allantoic route with allantoic passage of Enders strain of mumps virus in a dilution 10^{-3}. The diluent contained 0.067 M sodium potassium phosphate (*p*H 7.3) and 0.2 per cent of bovine albumine (Povient Producten N.V. Amsterdam). After five days of incubation at +36.0°—36.5° C the infected eggs were chilled overnight in the icebox. The following day the allantoic fluid was collected under sterile conditions, centrifuged at 1500 r.p.m. for 10 minutes, and dialysed against phosphate buffer at +4° C for 48 hours. After a second centrifugation, of a similar kind, Merthiolate (Eli Lilly & Company) 1/10,000 and phenol 1/1,000 was added. The antigens were stored at + 4° C; they showed no measurable variation in hemagglutinating titer during one month's storage. Each lot of antigen was titrated with convalescent phase antiserum using the Lederles mumps virus antigen of the viral type as a standard. The dilution of antigen used in the series, usually ½, was that giving the same titer as the Lederles antigen.

In this connection it may be mentioned that a mumps virus strain, isolated in Finland by one of us (Penttinen), gave as antigen in a complement fixation test similar results with five convalescent phase sera as the two above-mentioned antigens. This virus was adapted to grow in the allantoic cavity after 8 transfers in the amniotic sac. The antigen was made from the 20th egg passage as described above. The results of the complement fixation test suggest that the antigenic variations of mumps viruses in different epidemics are small. The results obtained by Lundbäck and Hook *et al.* (7, 10) seem to point to the same direction.

The total volume of reagents in each tube in titrations and controls was the same — 1.0 ml. The volume of each reagent pipetted into the tubes was always the same — 0.2 ml. As a diluent and inactive reagent, 0.9 per cent sodium chloride solution was used throughout.

[1] The children's sera were collected by Dr. Anneli Ylinen, to whom we wish to express our thanks.

[2] The patients' sera were collected at the Helsinki City Epidemiological Hospital, Director Professor Viljo Rantasalo, M.D., and at the Childrens Clinic, Director Professor Arvo Ylppö, M.D., We are indebted to Professors Rantasalo and Ylppö for their courtesy.

Hemolytic Amboceptor. — The amboceptor used in our laboratories for Wassermann tests was titrated with complement dilution 1/15 in dilution series of 1/1000, 1/2000, 1/3000, 1/4000, 1/6000, 1/8000 and 1/10,000. The dilution of amboceptor in complement titration and in the test proper was twice the last dilution showing complete hemolysis.

Complement. — Pooled quinea-pig complement was titrated in the presence of antigen with a dilution factor of 1.26, beginning at a dilution of 1/10. The dilution of complement used in the serum and antigen controls was the last one showing complete hemolysis in complement titration, and in the test proper 1.26 times that dilution.

Cells. — 2.5 per cent washed sheep cells were used in all titrations.

Sera. — The sera were stored at −18° C until used and inactivated at +56° C for half an hour before testing. The dilution factor was 2 in the titrations and the first dilution ¼.

The Test Proper. — 0.2 ml of serum dilution, of antigen, and of complement in proper dilutions were incubated at +37° C for 45 minutes. Fifteen minutes before the addition of the hemolytic system, equal amounts of cells and amboceptor dilution were mixed. To each tube 0.4 ml of sensitized cells was added and the incubation continued until the serum and the antigen controls showed complete hemolysis. The last serum dilution showing complete fixation was considered the titer of the serum.

Controls. — In all series, controls of the hemolytic system, the antigen and the serum were included, the dilution of the antigen being the same as in the test proper and the dilution of the serum ½.

To each series a titration of a known positive serum was added as a further control. Some variation in the titer of the same serum occurred in the different series, but not more than 1 tube up or down.

Normal allantoic fluid control was only used occasionally because, according to our opinion, the value of that control is very limited, due to the different protein contents of infected and uninfected allantoic fluid.

Hemagglutination Inhibition Technique. — The antigen was the same as in the complement fixation tests, and it usually gave a titer of 1/640. The pattern method with 0.5 per cent chicken cells and 1/32 as the first serum final dilution was used. The amount of virus used was 4 times that causing complete hemagglutination under the test conditions. Known positive serum was titrated in all series. The variation in titer of the same serum in different series was not more than one tube up or down.

THE RESULTS

CONTROL MATERIAL

Table 1. shows the results of the titrations with the children's and medical students' sera. As can be seen, the children's sera do not show any anti-mumps titers in the lowest dilutions used, neither in complement fixation nor in hemagglutination inhibition. This is

TABLE I.

TITERS IN CONTROL MATERIAL.

Groups of control material	Number of cases	Complement fixation titers						Hemagglutination inhibition titers					
		<4[1]	4	8	16	32	64	<32[2]	32	64	128	256	512
Children (11-17 months)	9	9						9					
Adults with negative history of mumps ..	19	11	1	4	3			14	2	2	0	1	
Adults with positive history of mumps ..	25	3	5	6	8	3		10	3	5	5	2	

[1] Reciprocals of initial dilutions of sera
[2] Reciprocals of final dilutions of sera

very probably due to the age of children (11—17 months). The possible antibodies received from the mother had disappeared and they had had no experience with the mumps virus. The children's sera are accordingly suitable for the detection of unspecific positive results which may be due to unsuitable techniques.

The sera of the medical students were grouped into two groups according to the history of mumps. As can be seen from the results, there is a correlation with the history of mumps, although the group with no history of mumps virus infection also shows antibodies against mumps antigen. This confirms the results of other

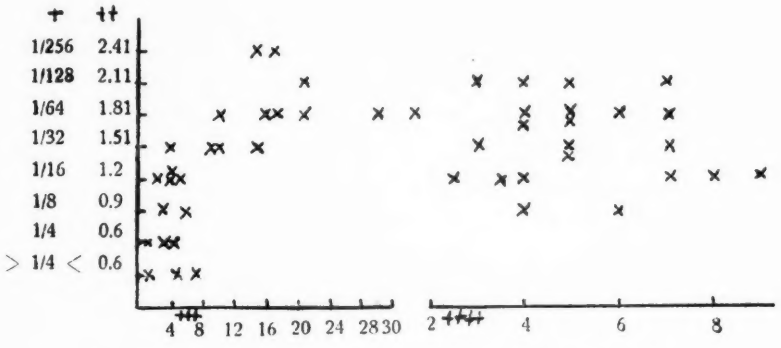

Fig. 1. — Complement fixation titers in cases of mumps.

\+ initial dilutions of sera
\+\+ logarithms of reciprocals of initial dilutions of sera
\+\+\+ days after onset of symptoms
\+\+\+\+ months after onset of symptoms

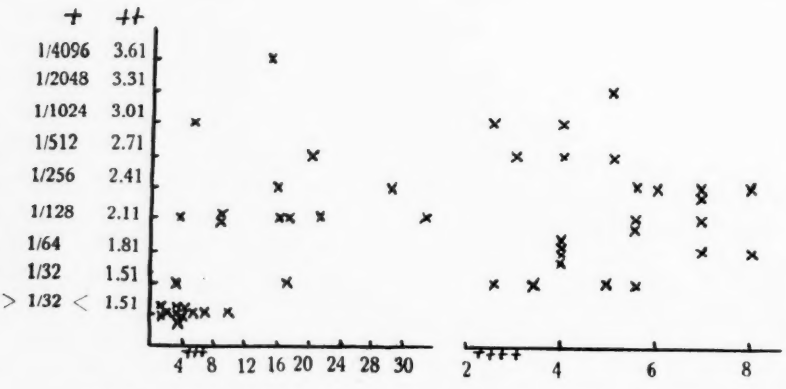

Fig. 2. — Hemagglutination inhibition titers in cases of mumps

\+ final dilutions of sera
++ logarithms of reciprocals of final dilutions of sera
+++ days after onset of symptoms
++++ months after onset of symptoms

investigators, that mumps virus infection often proceeds without typical clinical symptoms. If percentages from the small figures presented are estimated, it seems that about 90 per cent of the students in the group of positive history had measurable antibodies against mumps antigen, the corresponding figure in the group of negative history being about 40 per cent. According to Table 1, 75 per cent of Finnish medical students either have complement fixing antibodies against mumps antigen or give a history of mumps. With the hemagglutination inhibition technique the corresponding figure is 68 per cent. The insensitivity of our hemagglutination inhibition technique is indicated by the 10 cases with positive history and no antibodies measurable with the technique. This may be partly due to the unnecessarily high first tube dilution, which was selected according to Lundbäck (10), who showed that more than sixty per cent of his control sera without periodate treatment showed an inhibition titre of 1/512 or more.

SEROLOGIC TITERS AFTER INFECTION

In Fig. 1 the results of 47 complement fixation titrations of sera collected from 23 clinically certain mumps cases are plotted. All 47 specimens were titrated on the same day with the same system.

It can be seen that the titers generally rise rapidly, reaching the highest values 2—3 weeks after the onset of the symptoms and remain at markedly elevated levels, in some cases at least for 7 months.

According to Fig. 1 it seems, in most cases, to be possible to make a rapid serologic diagnosis of parotitis with the complement fixation test, if the first serum specimen can be collected right at the onset of symptoms. This is in good conformity with the observations of Henle, Harris, and Henle (4), who showed that antibodies against the soluble parotitis antigen are formed rapidly at the beginning of the disease. Our antigen contained all non-dialysable substances of infected allantoic fluid and accordingly also the soluble antigen. Our results also support the conclusion of Aikawa and Meicklejohn (1), who were of the opinion that by separation of S and V antigens little practical profit can be obtained.

The results of hemagglutination inhibition tests are presented in Fig. 2. All 49 specimens from 23 cases of parotitis were titrated on the same day with the same system. It can be seen that of 16 specimens collected before the 12th day of the disease, 11 gave negative results in the lowest dilution tested. Partly this may be due to the above-mentioned unnecessarily high serum dilution in the first tube of the titrations and possible partly to our technique, where sera were not incubated with the virus before the addition of cells, as Aikawa and Meicklejohn did (1). The hemagglutination inhibition titers seem to remain at elevated levels at least 8 months and have in no case in the observation period declined below the the lowest dilution used. It can be said that with our technique the hemagglutination inhibition was not as suitable as complement fixation for a rapid diagnosis of mumps virus infection. According to our experiences, however, the testing of paired sera is a useful procedure even with the hemagglutination inhibition technique, if no rapid diagnosis is in question.

SUMMARY

Sera from 9 children, 44 medical students, and 23 cases of parotitis were tested with mumps virus antigen with both the complement fixation and hemagglutinatination inhibition technique.

1. The children's sera showed no antibodies against mumps.

2. Seventy-five per cent of the medical students either had complement fixing antibodies in their sera or gave a history of mumps.

3. Complement fixation titers of the sera in the cases of parotitis rose rapidly at the beginning of the disease and remained at markedly elevated levels, in some cases at least for 7 months.

4. The hemagglutination inhibition titers rose more slowly than the complement fixation titers, but they also remained at elevated levels in the observation period of 8 months.

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FROM THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY AND THE DEPARTMENT OF BOTANY, UNIVERSITY OF HELSINKI

ON PHYTAGGLUTININS PRESENT IN MUSHROOMS

By

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Factors capable of agglutinating red blood corpuscles have long been known to exist in the vegetable kingdom. Of the fungi in which similar factors are present e.g. the following have been the subject of earlier studies: *Agaricus campestris*, *Boletus edulis*, *Cantharellus cibarius* (2), *Amanita muscaria*, *Armillaria mellea*, *Tricholoma album*, *T. nudum*, *T. vaccinium*, etc. (5). Some of them have been found capable of agglutinating or hemolyzing the erythrocytes of different animal species in differing ways. This phenomenon has also been recommended for the differentiation of certain fungal species difficult to distinguish from one another.

Renkonen (18) was the first to show that certain phytagglutinins (*Cytisus cessilifolius*, etc.) are capable of specific agglutination of the human red corpuscles of different blood groups. The purpose of the present investigation has been to study the possible existence of species in the fungal flora that are in possession of similar qualities.

N. Malmström, of the Department of Botany, Helsinki University, has identified the fungi; J. Elo and E. Estola of the Department of Serology and Bacteriology of the same university have carried out the serological investigations.

MATERIAL

The material has been collected in the course of the investigation. Hence, we have had fresh material at our disposal. The

majority of the mushrooms were collected in the area of the City of Helsinki. Some came from Esbo Commune (in Nylandia) and some from Vihti Commune (within Regio Aboënsis).

N. Malmström has preserved the studied mushrooms. The dry specimens have been added to the fungus herbarium of the Botanical Museum. Some few species collected previously from the same localities were not retained, and in a couple of other cases the scanty material did not suffice for preservation.

TECHNIQUE

The mushrooms were ground fine in a mortar while fresh, and extracted in saline solution to a 10 per cent suspension. The suspension was allowed to stand for one hour at room temperature, and centrifuged at 3,000 r.p.m. for 10 minutes. In the periods between the investigations the extract was kept in a deep-freezer (−20° C). The micro-method was employed in the tests: 0.01 cc of the extract to be studied and an equal amount of 2 per cent red corpuscle suspension were placed in a 50 × 4 mm test tube. The tube was allowed to stand for one hour at room temperature, after which the result was read off microscopically. Fresh, washed red corpuscles of blood groups $A_1MRh + P +$, $A_2MRh + P +$, $BMNRh + P +$ and $OMNRh + P +$ served as test cells. Each fungal species was tested in saline dilutions 1/1, 1/3, 1/9, and the agglutinating or hemolyzing species were controlled titrating the agglutinins up to the titre limit in powers of −2.

RESULTS

Table 1 gives the fungi in alphabetic order by genera, and indicates the number of tested samples and the number of agglutinating, non-agglutinating and hemolyzing samples.

DISCUSSION

In previous experiments (2, 5) the lability of the phytagglutinins present in fungi has already been evidenced. Agglutination easily passes over into hemolysis. The specimens of one and the same species collected at different times and from different sites could vary in the way they reacted to certain animal blood corpuscles, sometimes yielding agglutination, sometimes hemolysis or non-

TABLE 1

Name of Fungus	Number of Tested Samples	Number of Samples Giving					
		Agglutination				Hemolysis	
		Titre $\geq 1/16$	Titre $= 1/2$-$1/8$	Titre $= 1/1$	No Reaction	Titre $\geq 1/8$	Titre $\leq 1/4$
Hymenomycetes							
Nomenclature principally according to Karsten (9, 10), supplemented with other authors listed in the bibliography (1, 7, 8, 11, 12, 13)							
Amanita mappa (Batsch) Fr.	1				1		
—»— muscaria (L.) Fr.*	16	16					
—»— muscaria forma aureola (Kalchbr.)	2	2					
—»— porphyria (Alb. & Schw.) Fr.	1				1		
—»— rubescens (Pers.) Fr.	1						1
Armillaria mellea (Vahl.) Fr.	2				2		
Bjerkandera amorpha (Fr.) Karst. = Leptoporus amorphus (Fr.) Quél.	1				1		
Boletus bovinus Fr.	2				2		
—»— edulis Bull.	1	1					
—»— elegans Schum.	2				2		
—»— luteus L.	1				1		
—»— piperatus Bull.	1				1		
—»— scaber Bull. var. niveus Fr.	1				1		
—»— variegatus Swartz.	2				2		
—»— versipellis Fr.	1				1		
Calocera viscosa (Pers.) Fr.	1				1		
Camarophyllus virgineus (Wulf.) Fr.	1						1
Cantharellus aurantiacus (Wulf.) Fr.	4				2		2
—»— umbonatus (Pers.) Fr. = C.muscoides (Wulf.) Karst.	3				3		
Clavaria flava Schaeff.	1				1		
—»— ligula Schaeff.	3				3		
Clitocybe aggregata (Schaeff.) var. sphaerospora Lange	1		1				
—»— angustissima (Lasch) Fr.	1						1
—»— clavipes (Pers.) Fr.	2	1			1		
—»— connata (Schum.) Fr.	1				1		
—»— diatreta Fr.	1					1	

*) for more detailed discussion vide infra.

Name of Fungus	Number of Tested Samples	Number of Samples Giving					
		Agglutination			No Reaction	Hemolysis	
		Titre $\geq 1/16$	Titre $= 1/2$-$1/8$	Titre $= 1/1$		Titre $\geq 1/8$	Titre $< 1/1$
Clitocybe ditopus Fr.	1						1
—»— infundibuliformis (Schaeff.) Fr.	1				1		
—»— nebularis (Batsch) Fr.	1	1					
—»— obsoleta (Batsch) Fr.	2		2				
—»— odora (Bull.) Fr.	1				1		
—»— rivulosa (Pers.) Fr.	1					1	
Clitopilus orcella (Bull.) Fr.	1				1		
—»— prunulus (Scop.) Fr.	2				2		
Collybia butyracea (Bull.) Fr.	5					5	
—»— maculata (Alb. & Schw.) Fr.	1				1		
—»— platyphylla (Pers.) Fr.	1						1
—»— velutipes (Curt.) Fr.	1					1	
Coprinus atramentarius (Bull.) Fr.	1				1		
—»— comatus (Schum.) Fr.	2				2		
—»— micaceus (Bull.) Fr.	1				1		
Cortinarius anomalus Fr.	1				1		
—»— armillatus Fr.	1				1		
—»— brunneus (Pers.) Fr.	5		2		3		
—»— camphoratus Fr.	1	1					
—»— cinnamomeus (L.) Fr.	4			2	2		
—»— collinitus (Pers.) Fr.	2				2		
—»— elatior Fr.	2				2		
—»— flexipes Fr.	1				1		
—»— fulgens (Alb. & Schw.) Fr. sensu Cooke (Lange 1938)	1				1		
—»— gentilis Fr.	2				2		
—»— imbutus Fr. var. vilior Karst.	1				1		
—»— obtusus Fr.	1			1			
—»— semisanguineus Fr.	3				3		
—»— traganus Fr.	1				1		
—»— triumphans Fr.	2				2		
Deconica bullacea (Bull.) W.Sm. = Psilocybe bullacea (Bull.) Fr.	1	1					
Flammula alnicola Fr.	1	1					
—»— flavida (Schaeff.) Fr.	1						1
Gomphidius glutinosus (Schaeff.) Fr.	1				1		
—»— viscidus (L.) Fr.	1				1		

Name of Fungus	Number of Tested Samples	Number of Samples Giving					
		Agglutination				Hemolysis	
		Titre $\geq 1/16$	Titre $= 1/2-1/8$	Titre $= 1/1$	No Reaction	Titre $\geq 1/8$	Titre $1/4$
Hansenia hirsuta (Wulf.) Karst. = Coriolus hirsutus (Wulf.) Quél.	1				1		
Hebeloma crustuliniforme (Bull.) Fr.	1						1
Hygrophorus eburneus (Bull.) Fr. = Limacium eburneum (Bull.) Fr.	1	1					
—»— hypothejus Fr. = Limacium hypothejum Fr.*	7	7					
Inocybe geophylla (Sow.) Fr. var. alba Lange	1				1		
Inonotus radiatus (Sow.) Karst. = Xanthochrous radiatus (Sow.) Pat.	1				1		
Laccaria laccata (Scop.) Cook. var. amethystina Bolt.	4	4					
—»— laccata var. proxima Boud.	1				1		
Lactarius deliciosus (L.) Fr.	1				1		
—»— glyciosmus Fr. sensu Knauth & Neuhoff = L. confusus Lundell	1				1		
—»— rufus (Scop.) Fr.	4		2				2
—»— subdulcis (Pers.) Fr.s. Karst. = L. thejogalus (Bull.) Fr. s. Knauth & Neuhoff	1	1					
—»— torminosus (Schaeff.) Fr.	3	3					
—»— turpis (Weinm.) Fr.	1			1			
—»— vietus Fr.	1			1			
Lenzitina saepiaria (Schaeff.) Karst.	1				1		
Lepiota amianthina (Scop.) Fr.	3				2	1	
—»— carcharias (Pers.) Fr.	3				2		1
—»— clypeolaria (Bull.) Fr.	1				1		
—»— cristata (Alb. & Schw.) Fr.	1				1		
—»— granulosa (Batsch) Fr.	1				1		
—»— leucothites Vitt.	1				1		
—»— procera (Scop.) Fr.	1				1		
Marasmius oreades (Bolt.) Fr.*	1		1				
—»— peronatus (Bolt.) Fr.	1				1		
—»— scorodonius Fr.	1				1		
Mycena alcalina Fr.	1		1				
—»— epipterygia (Scop.) Fr.	2				2		
—»— galericulata (Scop.) Fr.	2			1	1		
—»— pura (Pers.) Fr.	1				1		

* for more detailed discussion vide infra

Name of Fungus	Number of Tested Samples	Number of Samples Giving					
		Agglutination			No Reaction	Hemolysis	
		Titre $\geqslant 1/10$	Titre $= 1/2$–$1/8$	Titre $= 1/1$		Titre $\geqslant 1/8$	Titre $\leqslant 1/4$
Naematoloma capnoides (Fr.) Karst.	2				2		
—»— sublateritium (Schaeff.) Karst.	1			1			
Naucoria scolecina Fr.	1						1
Omphalia campanella (Batsch) Fr.	1		1				
Paxillus involutus (Batsch) Fr.	3	3					
Pholiota mutabilis (Schaeff.) Fr.	3				3		
—»— squarrosa (O.F. Müll.) Fr.	3	3					
Piptoporus betulinus (Bull.) Karst. = Ungulina betulina(Bull.) Pat.	1				1		
Pleurotus ulmarius (Bull.) Fr.	1			1			
Pluteus cervinus (Schaeff.) Fr.	1				1		
Polyporellus brumalis (Pers.) Karst. = Leucoporus arcularius (Batsch) Quél. ..	1				1		
Polyporus ovinus (Schaeff.) Fr.	1	1					
Polystictus Schweinitzii Fr. = Phaeolus Schweinitzii (Fr.) Pat.	1				1		
Psalliota hortensis Cooke coll.	3	3					
Psathyra fusca (Schum.) Lange	1				1		
Psilocybe spadicea Fr.*	8	8					
Russula delica Fr.	1						1
—»— emetica (Schaeff.) Fr.	2			1			1
—»— fragilis (Pers.) Fr. = R. Mairei Singer (Lange 1940)	2			2			
—»— paludosa Britz. = R. elatior Lindbl.	1				1		
—»— puellaris Fr.	1				1		
—»— vesca Fr.	1			1			
Sarcodon imbricatus (L.) Quél.	1				1		
Sparassis crispa (Wulf.) Fr.	1				1		
Stereum hirsutum (Willd.) Fr.	1				1		
Stropharia aeruginosa (Curt.) Fr.	4		2		2		
—»— albocyanea (Desm.) Fr.	1				1		
Tyrodon repandus (L.) Karst.	1				1		
Trametes cinnabarina (Jacq.) Fr.	1				1		
Tricholoma albobrunneum (Pers.) Fr. = T. striatum (Schaeff.) Quél.	2		2				
—»— album (Schaeff.) Fr.	1				1		

* Fore more detailed discussion vide infra.

Name of Fungus	Number of Tested Samples	Number of Samples Giving					
		Agglutination			No Reaction	Hemolysis	
		Titre $\geq 1/16$	Titre $= 1/8$-$1/8$	Titre $= 1/1$		Titre $\geq 1/8$	Titre $\leq 1/4$
Tricholoma equestre (L.) Fr.	1			1			
—»— flavobrunneum Fr.	2			1			1
—»— grammopodium (Bull.) Fr.	1	1					
—»— imbricatum Fr.	1	1					
—»— nudum (Bull.) Fr.	2		2				
—»— pessundatum Fr.	1		1				
—»— rutilans (Schaeff.) Fr.	1				1		
—»— saponaceum Fr.	4	2					2
—»— vaccinium (Pers.) Fr.	2				2		
—»— virgatum Fr.	1				1		
Gasteromycetes							
Nomenclature according to Th. Fries(4)							
Bovista nigrescens Pers.	1				1		
Calvatia saccata (Vahl.) Morg.	1				1		
Lycoperdon perlatum Pers. = L.gemmatum Fr.	1				1		
—»— pyriforme Pers.	1				1		
Discomycetes							
Nomenclature according to W. Migula (14)							
Peziza aurantia Müller = Aleuria aurantiaca Fuck.	2	2					
Rhytisma acerinum (Pers.) Fr.	1				1		
Myxomycetes							
Nomenclature according to Rob. Fries (3)							
Lycogala Epidendrum (L.) Fr.	2				2		

agglutination. An explanation advanced is, that the fungi studied may have been closely related variants of the same fungus, or that the mode of reaction is an individual property in fungi. In addition it has been found that the toxicity of a fungus bears no correlation to the mode of reaction (5, 15, 16, 17).

The present investigations corroborated the above-mentioned observations. The agglutinins require test tubes washed with particular care, as otherwise they react easily with hemolysis.

Hemolysis was present almost constantly in high concentrations of strongly agglutinating fungal extracts. The lability of the agglutinins may have caused a hemolysing reaction of some fungus, although in other conditions it may yield agglutination or non-agglutination. No satisfactory explanation was found to the question.

A study of the localisation of the agglutinating factors in fungi revealed no essential difference between extracts made from the cap, gills or the stem.

Storage of fungus extracts in the deep-freezer (at −20° C) for 5 months produced no change in the reactions.

Of the 139 species studied, 36 species (26%) caused agglutination, 13 species (9%) hemolysis, and 78 species (56%) failed to react. Variations in the mode of reaction of the different specimens of one and the same species were found with 12 species (9%).

The reactions, in general, were unspecific. However, specificity to ABO system was present in certain species.

SPECIFICALLY AGGLUTINATING FUNGI

Hygrophorus hypothejus. — Titre values varied somewhat with the different specimens, but in every case the anti-B titre was 1—2 tubes longer than the anti-A_1 anti-A_2 titres. O-cells were not agglutinated.

52 samples of blood, including 24 of Group A, 11 of Group B, 14 of Group O and 3 of Group AB were investigated with the extract. None of the O bloods studied reacted, the others gave strong agglutination.

The fungal extract was absorbed as follows with the different kinds of cells: 2.0 cc of extract was placed in each of 4 tubes. 0.2 cc of washed, packed A_1 cells were added to one tube, and A_2, B and O cells to the others, respectively. The tubes were allowed to stand at room temperature for an hour, during which time they were turned up and down a few times. After that the contents of the tubes were centrifuged, and the supernatant fluid was titrated with all the cells in the usual manner. The results of absorption of H. hypothejus extract are given in table 2.

TABLE 2

Before Absorption				After Absorption by															
				A_1				A_2				B				O			
A_1	A_2	B	O	A_1	A_2	B	O	A_1	A_2	B	O	A_1	A_2	B	O	A_1	A_2	B	O
+++	+++	+++	—?	—	—	—	—	—	—	—?	—	—	—?	—	—	+++	++	+++	—?
++	++	+++	—	—	—	—	—	—	—	—	—	—	—	—	—	++	++	++	—
+	+	++	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	++	—
(+)	+	++	—	—	—	—	—	—	—	—	—	—	—	—	—	(+)	(+)	—?	—
—?	(+)	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—?	—?	—?	—
—?	—?	(+)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—?	—?	—?	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

The strength of the reactions is indicated by signs:

Positive: +++ one large clump, complete agglutination.
++ clear agglutination.
+ clumps just visible macroscopically.
(+) only microscopically visible agglutination.
Negative: —? traces of agglutination microscopically.
— microscopically homogenous suspension.

When unabsorbed the extract does not agglutinate O cells, while the other cells are agglutinated. After absorption by A_1, A_2 and B cells the fluid completely lost its activity. Absorption by O cells only changed the reactions slightly.

Psilocybe spadicea. — 8 different samples were available. In 5 of them the anti-A_1 titre was the longest, the anti-A_2 and anti-B titres 1—2 tubes shorter, and the anti-O titre 5—6 tubes shorter. In 3 specimens anti-A_1, anti-A_2 and anti-B titres were of equal length, and the O titre was 2—4 tubes shorter.

The original extract was diluted to 1/32 and 1/64. 182 blood samples, comprising 75 of Group A, 32 of Group B, 13 of Group AB and 62 of Group O were investigated with the extracts thus obtained. The samples of Group O could be distinguished from the others by the extracts. 127 of the samples were Rh positive and 55 Rh negative. The Rh factor was not found to affect the results.

Absorption with different cells was effected by adding to the extract washed, packed red blood cells, first amounting to 10 volume per cent of the total extract (Table 3). The absorbed extracts were then reabsorbed with 30 volume per cent of the same corpuscles (Table 4).

TABLE 3

Dilution	Before Absorption				After Absorption by															
					A_1				A_2				B				O			
	A_1	A_2	B	O	A_1	A_2	B	O	A_1	A_2	B	O	A_1	A_2	B	O	A_1	A_2	B	O
$^1/_1$	h	h	h	h	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
$^1/_2$	+++	+++	+++	+++	+++	+++	+++	+	+++	+++	+++	++	+++	+++	+++	++	+++	+++	+++	+++
$^1/_4$	+++	+++	+++	+++	+++	+++	+++	—	+++	+++	+++	—	+++	+++	+++	—	+++	+++	+++	(+)
$^1/_8$	+++	+++	+++	+	+++	+++	+++	—	+++	+++	+++	—	+++	+++	+++	—	+++	+++	+++	—
$^1/_{16}$	+++	+++	+++	—?	+++	++	++	—	+++	++	++	—	+++	+++	+++	—	+++	+++	+++	—
$^1/_{32}$	+++	+++	+++	—	++	+	(+)	—	++	+	+	—	+++	+	+	—	+++	+	++	—
$^1/_{64}$	+++	+++	++	—	—	—?	—?	—	(+)	—?	(+)	—	++	(+)	—	—	+++	+	(+)	—
$^1/_{128}$	+	+	(+)	—	—	—	—	—	—?	—	—	—	—	—	—	—	(+)	(+)	—?	—
$^1/_{256}$	(+)	(+)	—?	—	—	—	—	—	—	—	—	—	—	—	—	—	—?	—	—	—
$^1/_{512}$	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

h denotes hemolysis.

TABLE 4

Dilution	After Second Absorption by															
	A_1				A_2				B				O			
	A_1	A_2	B	O	A_1	A_2	B	O	A_1	A_2	B	O	A_1	A_2	B	O
$^1/_1$	—	—	—	—	+++	+	+++	—	+++	+	—	—	+++	+++	+++	—
$^1/_2$	—	—	—	—	(+)	—?	—?	—	(+)	—	—	—	+++	+++	+++	—
$^1/_4$	—	—	—	—	—	—	—	—	—	—	—	—	+++	++	++	—
$^1/_8$	—	—	—	—	—	—	—	—	—	—	—	—	++	+	—?	—
$^1/_{16}$	—	—	—	—	—	—	—	—	—	—	—	—	—?	(+)	—	—
$^1/_{32}$	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Both with *Hydrophorus hypothejus* and *Psilocybe spadicea* a distinct difference is noticeable between absorption with A_1, A_2 or B cells, on the one hand, and with O cells on the other. The absorptive ability of the former is considerably higher than that of the latter; the same applies to agglutination titres. A possible explanation for this may be the similarity in the structure of agglutinogens A_1, A_2 and B, differing from the structure of agglutinogen O. The position of A_2 cells especially seems interesting.

The similarities of groups A, B and AB (agglutinogen C) have already intrested some earlier workers (6, 19).

Marasmius oreades. — The extract of *M. oreades* seems to be unique in its content of anti-B.

TABLE 5

THE AGGLUTINATION OF MARASMIUS OREADES .

	$^1/_1$	$^1/_2$	$^1/_4$	$^1/_8$	$^1/_{16}$
A_1	—?	—	—	—	—
A_2	—?	—	—	—	—
B	+++	++	+	(+)	—
O	—?	—	—	—	—

As this single specimen only was available no further investigations could be carried out.

Amanita muscaria. — A fairly constant phenomenon found in the 16 specimens studied was that the reactions of anti-A_2 and anti-O were in all dilutions stronger than the corresponding reactions of anti-A_1 and anti-B. End titres were about equal.

SUMMARY

The agglutination ability of 10 per cent saline extracts made of fungi on A_1, A_2, B and O corpuscles was studied. Of the 139 species of fungi studied, 26 per cent agglutinated, 9 per cent hemolyzed and 56 per cent did not react. With 9 per cent, the modes of reaction varied.

Distinct specificity was observed with 2 fungi only, *Hygrophorus hypothejus* and *Psilocybe spadicea*. These agglutinated A_1, A_2 and B corpuscles with a considerable titre, but O corpuscles more weakly or not at all. The absorbtive activity of O cells, also, was weaker than that of the other cells. Hence it is possible that a struc-

tural similarity exists between groups A_1, A_2 and B, which is lacking in O cells.

The titre of the single available specimen of *Marasmius oreades* with B cells was distinctly longer than with other cells.

The common fly-bane, *Amanita muscaria*, gave a stronger agglutination with A_2 and O cells than with A_1 and B cells, but no distinct difference was ascertainable with regard to titre limits.

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FROM THE STATE SERUM INSTITUTE, HELSINKI

THE SITOLIPIN TUBE TEST IN CEREBROSPINAL FLUID EXAMINATION

By

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The Wassermann test, performed with both common and cholesterolized extract, has been used at this institute for cerebrospinal fluid diagnostics of syphilis. Both tests have been performed with two different amounts of cerebrospinal fluid. As the VDRL test has proved applicable to cerebrospinal fluid examinations (2) we have also studied the use of sitolipin antigen for this purpose (1).

The method used in the Sitolipin tube test is the same as in the corresponding VDRL test. The samples of cerebrospinal fluid tested have been mainly obtained from internal disease, venereal disease and psychiatric hospitals. The total number of samples was 324. Positive reactions were obtained in both the Wassermann test and the sitolipin tube test in 34 cases. Fifteen cases were negative in the Wassermann test but positive in the Sitolipin test, and three were positive in the Wassermann test but negative in the Sitolipin test.

WaR	Sitolipin Test	No. of Cases
+	+	34
−	+	15
+	−	3
−	−	272
	Total	324

In seven of the fifteen cases which were sitolipin positive but Wassermann negative the hospital diagnosis was encephalomeningitis or myelo-encephalomeningitis luetica, in four cases tabes dorsalis, in three cases lues medicata seropositiva, and in one case lues III, mesaortitis luetica.

In the three cases with a positive Wassermann reaction but a negative Sitolipin test the hospital diagnosis was, respectively, lues cerebrospinalis, tabes dorsalis and anophthalmia oculi dx. In the last mentioned case a new examination made three weeks later gave negative Wassermann and sitolipin reactions.

These results indicate that the use of Sitolipin in VDRL tube tests of cerebrospinal fluid has proved more responsive than the WaR and also highly specific, as syphilis was present in all the cases with a positive sitolipin reaction. The patients had syphilis in two of the three cases in which the WaR was positive but the sitolipin test negative, but in the third case no indication of syphilis was found other than a positive WaR, which, however, became negative in a later test. The positive reaction in this case should therefore be regarded as non-specifically positive.

I wish to add that we have only recently used Sitolipin antigen in the complement fixation test of cerebrospinal fluids. In addition to its possibly greater sensitivity I hope it will also possess the advantage of a smaller self-fixation action on the complement.

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FROM THE STATE SERUM INSTITUTE, HELSINKI

THE TITER VALUES OF THE VDRL TUBE TEST WITH SITOLIPIN AS THE ANTIGEN

By

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In our previous investigations (1), (2), (3), we have found that Sitolipin, isolated from wheat germs, is a very specific and useful syphilis antigen, somewhat more sensitive that the standard Kahn test. For the last-mentioned reason, it would seem likely that when Sitolipin is used as the antigen the titre values obtained will be higher than given by the Kahn test.

We have carried out the titration of 250 syphilis sera to study the possible difference in the above titre values. We used Kahn antigen and Sitolipin antigen in the studies. With the Kahn antigen we effected a standard Kahn test and with Sitolipin a VDRL tube test (4). It may be mentioned that the sensitivity of the Kahn test as used in our institute was compared, in the Spring of 1950, with the Kahn test employed by the VD Research Laboratory in New York and was found to be practically of the same level of sensitivity.

A review of the results we attained in the way mentioned earlier showed that the VDRL test with Sitolipin used as the antigen, generally yields a higher titre value than the Kahn test. Of the total material of 250 syphilis sera, 215 or 86% were cases in which the titre yielded by Sitolipin was higher than the Kahn titre, and among a considerable part of these cases the difference in titre was double or higher. Of the remaining thirty-five cases, 24 instances or 9.6% gave the same titre values for Kahn and Sitolipin, while in

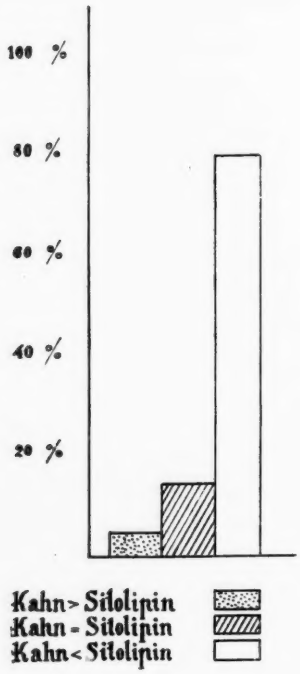
100 %
80 %
60 %
40 %
20 %
Kahn > Silolipin
Kahn = Silolipin
Kahn < Silolipin

Fig. 1

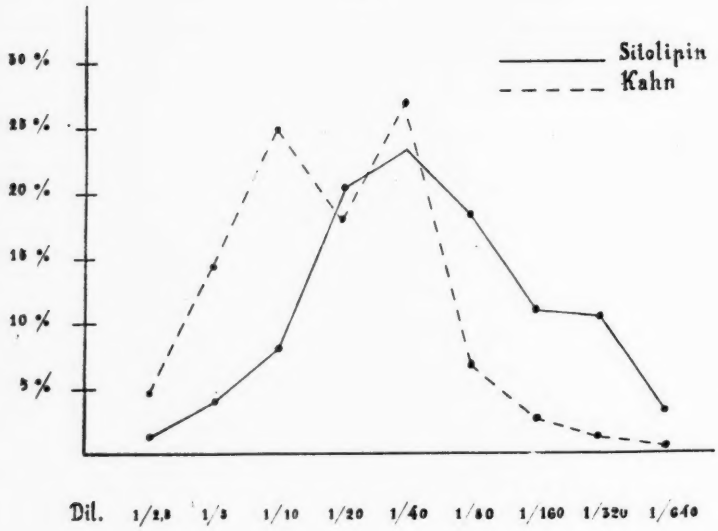
30 %
25 %
20 %
15 %
10 %
5 %
Silolipin
Kahn
Dil. 1/2,5 1/5 1/10 1/20 1/40 1/80 1/160 1/320 1/640

Fig. 2

11 cases, or 4.4%, the Kahn titre was higher than the Sitolipin titre. In the last-mentioned cases, apart from one, the difference in titre values did not exceed one step.

Fig. 2 shows that, as mentioned previously, the titre values of Sitolipin generally are higher than the titre values of Kahn, and further that in the majority of cases the difference in titre values is double, or one step higher when the VDRL tube test with Sitolipin as the antigen is used than when the Kahn test is employed. In addition, Fig. 2 shows that relatively high titre values are considerably more frequent using the VDRL tube test with Sitolipin as the antigen than with the standard Kahn test.

It may be mentioned that, in addition to the above studies, we have effected parallel titrations with Cardiolipin and Sitolipin in the VDRL tube test with 64 syphilis sera. The results attained in these studies have been practically entirely comparable. Of these 64 cases, 57 or 89% gave the same titre values with both antigens, while in 7 cases or 11% the titre values obtained differed. Out of these 7 cases, the titre with Cardiolipin was higher than that with Sitolipin in three cases, while in 4 cases the contrary result was obtained. It may be mentioned that in these 7 cases in which the titre values differed, in no single instance did the difference exceed one step.

In addition to the results obtained in the studies reported on above, it may be justifiable to mention that when the VDRL tube test was used in the titrations, with either Cardiolipin or Sitolipin as the antigen, the reading of results was very easy and clear, for the dividing line between positive and negative was generally so sharp that practically no error was possible in the reading of the results. This difference between positive and negative, in our experience, is perhaps somewhat more typical still of Sitolipin than of Cardiolipin.

To summarise the studies effected, it may be said:

— the VDRL tube test, with Sitolipin as the antigen, as a rule gives higher titre values in the titration of syphilis sera than the Kahn test. Although, in the majority of cases, the in titre values are double only, but quite a considerable number of cases yields remarkably higher differences in titres;

— the VDRL tube test, with Cardiolipin and Sitolipin used as antigens, yields results practically mutually corresponding.

— Sitolipin has proved a very serviceable antigen when syphilis sera are titrated by means of the VDRL tube test. The distinction between a positive and a negative reaction has proved to be clear and easy to read.

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RESULTS OBTAINED WITH VDRL SLIDE FLOCCULATION TEST WITH SITOLIPIN AS THE ANTIGEN

By

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Having previously (1), found that Sitolipin, isolated from wheat germs, was a useful, relatively specific and sensitive syphilis antigen in the VDRL (3) tube test, we carried out investigations into the suitability of Sitolipin antigen for use in the VDRL slide test as well.

To this end we examined 9830 sera obtained from different hospitals and policlinics, comparing the results yielded by Sitolipin in the VDRL (4) slide test with the results yielded by the Kahn test, and in 1630 cases with results obtained in the VDRL slide test using Cardiolipin as the antigen.

TABLE 1

Kahn	Sitolipin	Number of Cases
+	+	132
+	—	19
—	+	32
—	—	9647
Total number of cases		9830

Out of the 9830 samples examined, 9647 gave a negative finding with both the Kahn test and the Sitolipin VDRL slide test. With 132 samples of the material examined both the Kahn and the Sitolipin VDRL slide test were positive, in 19 cases Kahn only

was positive, and with 32 samples the Kahn test was negative but the Sitolipin VDRL slide test was positive. It may be mentioned that in all cases in which the results differed a VDRL slide test with Cardiolipin as the antigen was also carried out, and in this investigation we found no single case in which the results obtained with Sitolipin and Cardiolipin differed.

In the clinical examinations carried out it was found that out of the 19 cases in which the Kahn test alone was positive, in 18 nothing indicative of syphilis could be found, whereas previous syphilis was found in one case and treatment had been discontinued two years earlier. According to the clinical diagnosis, the breakdown of these 18 cases was: scarlatina 7 cases, pneumonia 2 cases, parotitis 3 cases, endocarditis lenta 2 cases, paratyphoid 1 case, bronchitis chr. 2 cases and nihil obj. 1 case.

In 28 of the 32 cases in which sitolipin alone was positive, something distinctly indicative of syphilis could be found (cases of syphilis under treatment or treated earlier), whereas in four cases there was nothing definitely indicative of syphilis. The breakdown of these four cases was: Graviditas 2 cases, Asthma bronchiale 1 case, and urethritis gonorrhoica 1 case.

As is obvious from the above, 19 of the 9,830 cases examined would have remained undetected if the samples had been investigated by the Sitolipin — VDRL test alone; however, one of them only was a fairly definite case of syphilis while 18 were unspecific positive results. If the Kahn test alone had been used, 28 cases of syphilis would have remained negative, and in 4 cases only we would have obtained a positive result in cases in which nothing distinctly indicative of syphilis could be found.

In addition to the above investigations, we have carried out simultaneous VDRL slide tests of the same samples with 2,362 cases using both cardiolipin and sitolipin as the antigens. In none of the examined cases were the results obtained completely different from one another, although in some cases one of the reactions was distinctly stronger than in the others. Cases of this kind totalled 5, and in two of them cardiolipin yielded a more distinct positive result than Sitolipin, while in one Sitolipin was more strongly positive than Cardiolipin.

The last-mentioned results differ somewhat from the first preliminary investigation results, in which we e.g. compared the

results yielded by Sitolipin and Cardiolipin using the VDRL (2), tube test, but at that time we still had not at our disposal a Sitolipin meeting the requirements we demanded of it later on, nor lecithin that met the standards of purity demanded by Pangborn. However, it is possible and also probable that cases will occur in which differing results will be obtained with Cardiolipin and Sitolipin provided the investigation material is extensive enough.

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A NEW ANTIGEN FOR SYPHILIS ISOLATED FROM BEEF HEART

By

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The so-called pure syphilis antigens known to date, viz. Cardiolipin (1—2), and Sitolipin (4), are both nitrogen-free, unsaturated compounds containing phosphorus. Judging by these two substances, which in their chemical and biological properties greatly resemble one another in spite of the fact that they are isolated from entirely different raw materials, it could be assumed that the antigen property of syphilis antigens lay just in such phosphorus compounds. However, this assumption is contradicted by the investigations which have led us to the possibility of isolating from beef heart a new compound, active as syphilis antigen, also unsaturated, but free of phosphorus and containing nitrogen.

METHOD OF PREPARING THE ANTIGEN

Beef heart free from fat and connective tissue is ground and dried. The dried material is ground into fine powder. The powder is extracted with peroxide-free ether, using 400 ml of ether per 100 g of powder. The extraction is carried out at room temperature with careful agitation for 24 hours, after which the ether is decanted and the paste filtered. Then the extracting with ether is repeated for 24 hours, using 600 ml of ether per 100 g of powder.

The ether is decanted and the paste filtered until as dry as possible, after which the paste is spread e.g. on greaseproof paper and allowed to dry completely. The powder obtained is extracted,

using 500 ml of 98% ethanol per 100 g of powder. The extraction is carried out for 48 hours, with careful agitation all the time.

The alcohol solution is recovered by filtering, and the paste washed with a small amount of 98% ethanol. To the resulting completely clear alcohol extract, 20 % $BaCl_2$ solution is added, with careful continuous stirring, until a flocculating precipitate ceases to form. Care must be taken that no considerable surplus of $BaCl_2$ is used. Then filter and add 50% $CdCl_2$ solution to the clear filtrate, with careful continuous stirring, until no further precipitate forms. Allow to chill overnight.

The alcohol is decanted and the precipitate collected by centrifuging. A suspension is made of the precipitate in 80% ethanol saturated with petrol ether, and poured into a separatory funnel, after which an equal volume of petrol ether is added and the mixture is shaken carefully for some 15 minutes. (A suspension is made of the precipitate obtained from ten beef hearts in 400 ml of 80 % ethanol saturated with petrol ether.) A part of the precipitate remains undissolved. The petrol ether layer is withdrawn.

The petrol ether is evaporated in a vacuum and carbon dioxide atmosphere. The residue is dissolved in chloroform, and the solution is shaken several times with 30% ethanol until $CdCO_3$ precipitate can no longer be established in the alcohol layer with 10% Na_2CO_3.

The chloroform layer is withdrawn and the chloroform evaporated in a vacuum until dry. The evaporation residue is dissolved in 96% ethanol (the amount obtained from ten beef hearts is dissolved in 700 ml of 96% ethanol.) To the alcohol extract is added, with continuous careful agitation, 50 % $CdCl_2$ solution until no further flocculating precipitate forms. Allowed to chill overnight. The alcohol solution is decanted and the precipitate collected by centrifuging.

The precipitate is dissolved in 80% ethanol saturated with petrol ether, this time using only half the ethanol-petrol ether quantity as before. An equal volume of petrol ether is added and shaken carefully for some 15 minutes. The petrol ether layer is withdrawn.

The petrol ether is evaporated in a vacuum and carbon dioxide atmosphere until dry. The residue is dissolved in chloroform and shaken with 30% ethanol until no Cd can be indicated in the ethanol layer. The chloroform layer is withdrawn.

The chloroform is evaporated in vacuum until dry, and the chloroform-free residue is dissolved in absolute ethanol (the amount obtained from ten hearts is dissolved in 100 ml of ethanol, when the solution will contain approx. 1 mg of antigen per ml of ethanol).

The substance isolated from beef heart by the above method is unsaturated, the iodine number varying between 70—90. It contains approx. 2% of nitrogen, and is completely free from phosphorus. An alcohol solution, it is not serviceable as such as a syphilis antigen but requires the addition, as do cardiolipin and sitolipin, of both cholesterol and lecithin. The lecithin employed has been egg lecithin prepared by Pangborn's method (3). In the alcohol solution of the substance isolated, to which both lecithin and cholesterol have been added, a »silkenswirl» similar to that in cardiolipin and sitolipin antigens is formed. In the serological investigations carried out, we have used 0.025% of the substance isolated, 0.3% of lecithin and 0.9% of cholesterol.

TABLE 1

Sitolipin	N-antigen	Number of Cases
÷	÷	161
÷	—	9
—	÷	28
—	—	892
Total number of cases		1,150

Table 1 gives the results of 1150 investigated cases, for which a simultaneous VDRL slide test (5), was effected using as the antigen both sitolipin and the previously mentioned new antigen containing nitrogen (N-antigen). As can be seen from the table, in 161 out of the 1150 cases investigated altogether, both the tests were positive, in 9 Sitolipin only gave a positive result, and in 28 N-antigen only produced a positive reaction. These 161 were cases in which syphilis was demonstrated either in anamnesis or status, and the same applies to the 9 Sitolipin-positive cases. In all the 161 cases, incidentally, the Kahn test was also positive, and in 73 of them WaR was positive. In none of the 28 cases in which the N-antigen alone gave a positive result could anything

indicative of syphilis be found, but they included two cases of scarlatina in which the Kahn test was positive but became negative in a later check-up examination.

As is obvious from the above, the N-antigen yielded a fully negative result in 9 cases of syphilis and an unspecific positive reaction in 28 cases.

It is possible that the nitrogen-containing substance, free of phosphorus, isolated by us from beef hearts, is hardly a useful antigen for syphilis diagnostics, since it has so far proved to be remarkably unspecific; its keeping qualities are poor in alcohol solution together with lecithin and cholesterol; hence, the addition of these substances must be effected almost immediately before the use of the antigen. Furthermore, with the present method at least, the amount of antigen obtained from beef hearts is so small that is makes the antigen disproportionately expensive. On the other hand, the substance is of theoretical interest, indicating that the alcohol extract of beef hearts contains yet another substance, in addition to cardiolipin, that acts as a syphilis antigen. Broadly speaking, a feature common to cardiolipin, sitolipin and N-antigen is that they are unsaturated compounds, relatively slightly alcohol-soluble, the primary difference between them being that cardiolipin and sitolipin are nitrogen-free and contain phosphorus whereas N-antigen is a compound of phosphorus but containing nitrogen.

As the study of the serological properties of the substance isolated by us is based on a very limited range of experiments, it is difficult to say anything certain about its antigen properties, and it may be possible that the said substance might be one of the factors that may affect the unspecific reactions yielded by crude beef heart alcohol extracts, a question to which an answer can perhaps be found by continued investigations.

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FROM THE STATE SERUM INSTITUTE AND FROM THE HOSPITAL FOR CONTAGIOUS DISEASES IN HELSINKI

ON SITOLIPIN, WASSERMANN AND KAHN REACTIONS IN CERTAIN INFECTIOUS DISEASES

By

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The value of seroreactions in syphilis diagnostics depends above all on their specificity and sensitivity. Sitolipin (4, 5) was isolated at the State Serum Institute from wheat germ powder, and according to earlier investigations (6, 8) its sensitivity was considerably greater than that of the Kahn reaction. The specificity of Sitolipin antigen did not suffer from sensitivity, and has shown itself at least equal to that of Kahn reaction (7, 8).

As it is known that both the Wassermann and Kahn tests in many different infectious diseases yield unspecific or false positive results (1, 2), our intention has been to obtain some idea of the relationship of sitolipin-antigen to certain infectious diseases by comparing the sitolipin-VDRL tube test (3) with the Wassermann, cholesterol-Wassermann and Kahn reactions in use at our Institute.

The material was obtained from the Municipal Hospital for Contagious Diseases in Helsinki, and due to widespread scarlet fever epidemics, it is derived for the most part from scarlatina patients. A grand total of 1,448 unselected blood samples were investigated, their breakdown by the different diseases being that shown in Table 1.

Both the sitolipin-VDRL tube tests and the Wassermann, cholesterol-Wassermann and Kahn reactions were carried out simultaneously. The samples in which any of the above reactions

TABLE 1

BREAKDOWN OF THE INVESTIGATED SERA BY DIFFERENT DISEASES

Scarlatina	Pneumonia Pleuritis	Angina Difteria Mononucleosis	Paratyphoid Typhoid Dysenteria Gastroenteritis	Infectio acuta	Poliomyelitis	Meningitis Encecephalitis	Hepatitis Cholecystitis	Otitis Sinuitis	Morbilli Varicellae	Pyelitis Cystitis	Insufficientia Cordis Apoplexia Thrombosis	Pertussis Bronchitis	Varia	
1173	50	47	23	23	27	15	14	10	2	8	8	6	36	1448

was positive (+), were taken as positive. The grand total of such sera was 30. Their breakdown by the different diseases, the reaction results, and the results of subsequent check-up examinations are given in Table 2. The first control examinations were effected 2—6 days after the first sample, the average control period being approx. 30 days. In two cases check-up examinations could not be effected for various reasons.

Table 2 includes 20 patients under 15 years; 19 of them had scarlatina, and one rhino-pharyngitis and ichtyosis. Of these 20, one was Wassermann-positive, 8 were cholesterol-Wassermann positive, 19 Kahn positive and one sitolipin positive.

Check-up examinations were effected subsequently in 19 cases, but this was impossible in one case (Case No. 6). In all the 19 cases in which check-up examinations were made, all the above reactions, sooner or later, became negative. In addition, the mothers were examined in 16 cases, in one the mother was dead, and three mothers were not available for control examinations. Accurate anamneses were made of the mothers, but only one of them had had syphilis in their anamneses, and with all the mothers the seroreactions carried out were negative, except one. In general, almost all the mothers had several healthy children, none of them had had stillbirths. Two mothers had had spontaneous abortions, one of them had one between two normal deliveries, the other had two at an early stage of pregnancy prior to the first delivery. Two mothers had previously had gonorrhea, but it had been cured without complications. One of them had been treated for gonorrhea at the policlinic of the Women's Clinic with sulfa drugs, and the other with penicillin at the Venereal Diseases Policlinic.

TABLE 2

POSITIVE RESULTS AND THEIR BREAKDOWN BY THE DIFFERENT DISEASE GROUPS, AND CHECK EXAMINATION RESULTS

Case No.	Lab. No.	Age of Patient Years	Diagnosis	WaR	Chol. WaR	Kahn	Sitol.	Mother WaR	Mother Chol. WaR	Mother Kahn	Mother Sitol.	Remarks
1	59588/50	1	Scarlat. [1]	—	+	+	—	—	—	—	—	
				—	—?	—?						
				—	—	—	—					
2	59789/50	10	»	—	—	+	—	—	—	—	—	
				—	—	—	—					
3	60950/50	7	»	—	—	+	—	—	—	—	—	
				—	—	—	—					
4	61526/50	4	»	—	—	+	—	—	—	—	—	
				—	—	+	—					
				—	—	—	—					
5	61877/50	13	»	—	—	+	—	—	—	—	—	
				—	—	—	—					
6	62249/50	5	»	—	+	+	—					
7	63926/50	9	»	—	—	+	—	—	—	—	—	
				—	—	+	—					
				—	—	—	—					
8	65550/50	12		—	—	+	—					Mother dead
				—	±	+	—					
				—	—	—	—					
9	65559/50	9	»	—	—	+	—	—	—	—	—	
				—	—	+	—					
				—	—	—	—					
10	66174/50	5	»	—	—	+		—	—	—	—	
				—	—	+	—					
				—	—	—	—					
11	66800/50	10	»	—	—	+	—	—	—	—	—	
				—	—	—?						
				—	—	—	—					
12	67067/50	7	»	—	—?	+		—	—	—	—	
				—	+		—					
				—	—	—	—					
13	67317/50	7	»	—	+	+	—	—	—	—	—	
				—	—	—	—					
14	69806/50	3	»	—	+	+	—	—	—	—	—	
				—	—	+	—					
				—	—	—	—					
15	71526/50	4	»	—?	±	+	—					
				—	—	+	—					
				—	—	—	—					

[1] Check-up examinations of each patient are given downward in chronological or

Case No,	Lab. No.	Age of Patient Years	Diagnosis	WaR	Chol. WaR	Kahn	Sitol.	Mother WaR	Mother Chol. WaR	Mother Kahn	Mother Sitol.	Remarks
16	71712/50	5	»	—	—?	+	—					
				—	—	—	—					
17	72713/50	2	»	—	—	—	+	—	—	—	—	
				—	—	—	—					
18	72907/50	10	»	—	+	+	—	—	—	—	—	
				—	+	—	—					
				—	—	—	—					
19	1372/51	6	»	—	+	+	—	+	+	+	+	
				—	—	—	—	+	+	+	+	Lues medicata by mother
				—	—	—	—					
20	63451/50	6	Rhinopharyngitis, Icthyosis	+	+	+		—	—	—	—	
				—	—	+	—	—	—	—	—	
				—	—	—	—					
21	65213/50	34	Poliomyelitis	—	±	+	—					
				—	—	+	—					
				—	—	—	—					
				—	—	—	—					
22	65392/50	50	»	—	—	+	+					Lues medicata
				—	+	+	+					
23	65216/50	25	Paratyphoid	—	—	+	—					
				+	—	+	—					
				± ±	+	+	—					
				—	—	+	—					
				—	—	—?	—					
				—	—	—	—					
24	67581/50	23	»	+	+	+	+					Lues recens
				+	+	+	+					
				+	+	+	+					
				+	+	+	+					
25	66586/50	28	Pneumon.	—	—	+	+					Lues medicata
				—	—	—?	+					
26	67337/50	51	»	—	—	+	—					
				—	—	+	—					
				—	—	+	—					
				—	—	±	—					
27	69369/50	53	Mesaortitis	—	—?	+	+					Lues III
				+	—	+	+					
				—	—	+	+					
28	71892/50	46	Angina	—	—	—	+					Lues medicata
				—	—	—	+					
29	119/51	42	»	+	+	+	+					Lues recens
30	1603/51	56	Pneumon.	—	+	—?	—					
				—	—		—					
				—	—	—	—					

It seems that the positive reactions in these 20 cases were due to the febrile disease from which the patient had suffered. It has in fact been reported previously that scarlatina especially gives such passing positive results (1). It is to be noted particularly that in 19 cases the sitolipin VDRL tube test was originally negative. In one case the sitolipin test only was positive and the other reactions negative.

10 of the patients in Table 2 were over 15. The youngest of them was 23 and the oldest 56. Of these 10 patients, four had a positive Wassermann, 5 a positive cholesterol-Wassermann, 8 a positive Kahn and 6 a positive sitolipin reaction. (The four Wassermann-positive were included in the four cholesterol-Wassermann positive, and these again in the 8 Kahn-positive reactions.)

Cases in which the sitolipin test was originally negative total 4. With 3 of these, all the said reactions could be proved negative in check-up examinations, with the fourth the Kahn test was ± after two months and the other reactions negative. Syphilis could be indicated, either in anamnesis or status, in none of these four patients.

With the six cases in which the sitolipin test was positive, in one all the other reactions effected were negative, but syphilis could be demonstrated in all of them. Four of the patients had lues medicata, one of them mesaortitis, and two had lues recens.

Table 2 shows a total of 7 positive reactions to the sitolipin VDRL tube test. One of them was positive temporarily only, and having appeared in the course of a passing febrile disease it must be considered unspecific. If we take the other reactions, Wassermann, cholesterol-Wassermann and Kahn as a whole, there were positive reactions in a total of 28 cases, including 23 of passing appearance, for which reason they should be considered unspecific or false positive results. In other words, the sitolipin test has given one unspecific reaction, whereas all cases of syphilis have been indicated. On the other hand, if Wassermann, cholesterol-Wassermann and Kahn reactions only had been used, one syphilis case would not have been detected, and in addition 23 unspecific or false positive results would have been obtained.

The whole material yielded positive reactions in approx. 2% of the cases, and of them 4/5 were unspecific.

SUMMARY

1. Using the method employed in the State Serum Institute, the sitolipin VDRL tube test and Wassermann, cholesterol-Wassermann and Kahn reactions were investigated simultaneously in the blood of 1448 unselected patients of the Municipal Hospital for Contagious Diseases in Helsinki.

2. Positive reactions were displayed by 30 patients, or approx. 2% of the total material. The necessary check-up examinations were made on 28 of them.

3. The sitolipin test was positive with 7 patients, 6 of whom had either treated or recent syphilis. The other reactions, Wassermann, cholesterol-Wassermann and Kahn tests, were positive with 28 patients, 5 of whom had either treated or recent syphilis; all these 5 cases were included in the above mentioned 6 sitolipin-positive syphilis cases.

4. The sitolipin VDRL tube test proved to be considerably more specific than the Wassermann, cholesterol-Wassermann and Kahn reactions, indicating all the syphilis cases and yielding only one unspecific result, while the other reactions, Wassermann, cholesterol-Wassermann and Kahn, failed to indicate one case of syphilis and yielded 23 unspecific results.

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STATE OF VITAMIN C NUTRITION IN ERYTHEMA EXSUDATIVUM MULTIFORME[1]

By

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During the Finnish-Russian war of 1941—44, attention was called to the frequent occurrence of erythema exsudativum multiforme among the troops stationed in the northernmost parts of Finland. This was thought to be due to a low state of vitamin C nutrition, particularly since good results were reported with vitamin C therapy. The question seemed to deserve careful attention. When the war was over, the present writers undertook to make a special study of the nutritional state of the body in vitamin C in cases of erythema exsudativum multiforme.

Baar (1) reported rapid recovery with ascorbic acid in two cases of moderate erythema exsudativum multiforme. In another case of the same condition, Hagemann (7) diagnosed vitamin C deficiency by dichlorophenol-indophenol titration in urine but did not say how much the deficiency amounted to. He treated the case by intravenous injections of ascorbic acid in daily doses of 200, 400, and 200 mg for three days and noted immediate recovery. Vitamin C preparations also gave good results in two other cases of erythema exsudativum multiforme with changes in the mucosa of the mouth

[1] The publication of this report was delayed by the writers' attempts to collect a considerably larger series of cases than that on which the report is actually based. The cases for the investigation were kindly placed at the writers' disposal by Professor P. Pirilä, M.D., Director of the Dermatological University Clinic, Helsinki. The vitamin C determinations were carried out in the University Institute of Medical Chemistry, Helsinki, by the kind permission of Professor P. E. Simola, M.D., Ph. D., Director of that Institute.

and in the lips, and in one case with changes in the mucous membranes only.

Lakaye and Lakaye (10) diagnosed deficiency of vitamin C by intravenous injections of ascorbic acid. Their estimations were based on the spontaneous vitamin C level in urine and not on a tolerance test. (The estimation of vitamin C was made by 2:6-dichlorophenol-indophenol titration.) Supposing that 20 mg per litre is a deficiency, they found that hypovitaminosis C was present in 15 cases out of 16. In one of these cases, however, the vitamin C level came near the limit value, being 18 mg per litre.

MATERIAL AND TECHNIQUE

The series examined consisted of 14 cases treated at the Dermatological University Clinic, Helsinki. An attempt was made to choose only cases of erythema exsudativum multiforme (bullous, pluriorificial) proper and those of the erythema exsudativum multiforme type caused by sulphonamide therapy. Cases of the latter type are, for instance, reported by Bickel (2) and Sonck (13). The majority of these cases, Nos. 1—8, belonged to the group described by Castello and Vandow (3), while Nos. 9—14 were caused by sulphonamide therapy. The symptoms are shown in table I.

The control series consisted of 43 persons of varying ages. In the women,[1] 18 in number, the serum vitamin C values ranged from 0.26 to 1.02 mg per 100 cc, with an average of 0.51 mg per 100 cc, and in the men, 25 in number, from 0.32 to 1.18 mg per 100 cc, with an average of 0.62 mg per 100 cc.

Vitamin C was estimated by the well-known 2:6-dichlorophenol-indophenol titration introduced by Tillmans (14). Serum analysis was carried out by a modification of the Farmer-Abt method (5) used in the University Institute of Medical Chemistry, Helsinki.[2] In urinalysis, titration was carried out in a milieu of 5 per cent acetic acid.

The blood specimens were taken in the morning on fasting subjects. As to the serum determinations during treatment, these

[1] In one case with the diagnosis erythema exsudativum multiforme or erythema nodosum determination of vitamin C in blood serum yielded the value 0.

[2] A detailed account of the method has been given by Pitkänen (11).

TABLE I

MATERIAL

No. of Case	Sex	Age, Years	Previous Occurrence of Dermatosis of the Same Type	Skin and Mucosal Symptoms					General Symptoms
				Pluriorificial	Bullous	Nodose	Mouth and Lips	Genitals	Headache
1	♀	54	often	+	−	−	+	+	+
2	♀	24	once	+	−	−	−	−	−
3	♀	42	»	+	−	−	−	−	−
4	♀	24	never	+	−	−	−	−	−
5	♀	25	»	+	−	−	−	−	−
6	♀	46	»	±	−	−	−	−	−
7	♀	56	»	+	−	−	−	−	−
8	♀	23	»	+	−	−	−	−	−
9	♀	37	»	−	−	+	−	−	−
10	♀	25	twice	+	−	−	−	−	−
11	♂	37	never	+	−	+	+	−	−
12	♂	35	once	+	−	+	+	+	−
13	♂	30	often	+	−	+	+	+	±
14	♀	11	never	+	+	+	−	−	−?

were made when at least 24 hours had elapsed since the last injection of ascorbic acid and at least 15 hours since the last intake of a 100 mg tablet of ascorbic acid. Serum analysis was made immediately after the withdrawal of the blood specimen. In order to eliminate any sources of error arising from hemolysis, Ivanow's pyramidon test was made in each case.

Twenty-five ml of glacial acetic acid was placed on the bottom of the bowl into which urine was collected; the analysis was carried out within 6 hours of the collection of the urine.

A number of control tests were carried out to check the methods used. Vitamin C added to the specimens could be estimated in 5 serum analyses and in 5 urinalyses, with an average accuracy of 4 per cent (the maximum error was 8—5 per cent). Titration of these urine specimens (and of those to which ascorbic acid had been added) was performed after a 24-hour storage in an ice box; the results were the same. This shows that small variations in the length of the time of storage could not affect the results.

RESULTS AND DISCUSSION

Table II shows that the state of vitamin C nutrition was lower in the cases of erythema exsudativum multiforme than in the control series.[1] It also shows that large intravenous injections of ascorbic acid rapidly raised the vitamin C level to normal. In all the cases the skin symptoms disappeared.

Whether vitamin C deficiency can be taken to be responsible for erythema exsudativum multiforme or to be a result of it is quite another matter. One thing that is rather striking is that in one case (No. 2) of erythema exsudativum multiforme the state of vitamin C nutrition was remarkably high. Hjärne (8) and Pitkänen (11) noted great seasonal variations in the nutritional state of the body in vitamin C. This might be accounted for by the fact that the threshold of vitamin C secretion varies from individual to individual (*e.g.*, Pitkänen). In general, every disease may result in hypovitaminosis C (Giroud and co-workers (6)). A fact speaking for the etiological significance of vitamin C deficiency is that it generally increases cellular permeability. In Finland, the incidence of erythema exsudativum multiforme is particularly high in late winter, when vitamin C nutrition is at its worst.

It seems probable, too, that there is some causal correlation between hypovitaminosis C and erythema exsudativum multiforme in those cases in which this condition follows sulphonamide medication (9). The administration of ascorbic acid reduces the toxicity of sulpha preparations both in animal and in clinical experiments. Diverging opinions, however, also occur (2, 4, 12).

Bickel (2) thinks that the factors responsible for any dermatosis following sulphonamide therapy are to be found either in *idiosyncrasy* to these drugs, in the actual toxic actions of these drugs, or in the combined action of light and the drug.

SUMMARY

1. In 14 cases of erythema exsudativum multiforme, vitamin C was estimated in the blood serum or in the urine. It ranged from 0.02 to 0.29 mg per 100 cc, with an average of 0.17 mg per 100 cc.

[1] Both in the present writers' and Pitkänen's series, examined by the same method in the University Institute of Medical Chemistry, Helsinki.

TABLE II

ESTIMATION AND DOSAGE OF ASCORBIC ACID

Case No.		Days 1	2	3	4	5	6	7	8	9	10	11	12	13	14	Remarks
1	Cb	0.02												0.96		0.96 suggests saturation. No reply to follow-up questionnaire.
	Cu	0	0	0	0	0	0	33	280		550			385		
	Th	300	300	300	300	300	300	500	500	500	500	500	500	500		
2	Cb	0.18						1.26								Follow-up: recurrence 6 months later, recovery with intravenous vitamin C therapy. No estimation of ascorbic acid. Estimation in urine at the end of therapy revealed a deficiency. High values in serum.
	Cu	1.5		3.5	7.5	8.5	20	40								
	Th	500	500	500	500	500	500	500								
3	Cb	0.23														Follow-up: dermatosis disappeared in a week. (Treatment exclusively with ascorbic acid.) No recurrence.
	Th	per os 100 mg three times daily for a week.														
4	Cb	0.25								0.42						Follow-up ca. 6 months later: recurrence, with 0.38 vitamin C in serum. The value was almost the same at the end of therapy, which shows that there was no deficiency in blood.
	Th	500	500	500	500	500	500									
5	Cb	0.08														Follow-up: the symptoms disappeared in a week, No recurrence.
	Th	per os 100 mg three times daily for a week.														
6	Cb	0.09														No reply to follow-up questionnaire.
	Th	per os 100 mg three times daily for a week.														

7	Cb	0.23														Follow-up: disappearance of symptoms in a week, No recurrence.
	Th	per os three times daily 100 mg for a week.														
8	Cb	0.20									0.40				0.38	No reply to follow-up questionnaire.
	Th	500	500	500	500	500	500	500	500	500	500					
9	Cb	0.1														Follow-up: disappearance of symptoms in a week, No recurrence.
	Th	per os 100 mg three times daily for ten days.														
10	Cb	0.23														No follow-up.
	Cu	1.5		3	25	55	110	220	360							
	Th	500	500	500	500	500	500	500								
		(Conditions simulating erythema exsudativum multiforme.)														
11	Cb	0.02														Follow-up: no answer to questionnaire.
	Cu	0	0													
	Th	300	300	300												
12	Cb	0.29														Follow-up: No recurrence; no use of sulphonamides.
	Th	500	500	500	500	500	recovery in 5 days.									
13	Cb	0.27														Follow-up: recurrence after the administration of sulpha drugs.
	Th	500	500	500												
14	Cu	0	0	120	140	140	—	110	35		75	—		44	25	Recovery in hospital. Follow-up: No ansever to questionnaire.
	Th	300	300	300	tabl. 1 × 3 for 12 days.											

Cb = estimation in serum: mg per 100 cc. Cu = estimation in urine: mg. per litre. Th = vitamin C therapy: intravenous injection, unless otherwise stated.

In a control series of 43 normal cases vitamin C was 0.26 to 1.02 mg per 100 cc (with an average of 0.51 mg per 100 cc) in women and 0.32 to 1.18 mg per 100 cc (with an average of 0.62 mg per 100 cc in men.

2. In 8 cases treated with intravenous injections or with oral administration of ascorbic acid daily, recovery took 7 to 10 days.

3. In 3 cases of allergic erythema exsudativum multiforme following sulphonamide therapy the nutritional state of the body in vitamin C estimated in blood serum was 0.29, 0.27, and 0.02 mg per 100 cc.

4. In all cases the skin symptoms disappeared with the improvement in the state of vitamin C nutrition.

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AUS DER MEDIZINISCH-CHEMISCHEN INSTITUT DER UNIVERSITÄT HELSINKI

ÜBER DIE ELEKTROPHORETISCH BESTIMMTE PROTEIN-KOMPOSITION UND LIPOIDGEHALT DES SERUMS SOWIE DIE FIBRINOGENMENGE DES PLASMAS BEI LEPRA

Von

RISTO PÄTIÄLÄ und TAPIO RAEKALLIO

(Der Schriftleitung zugegangen am 14 Febr. 1951

Von den 8 Patienten, welche im Frühjahr 1948 in Finnlands einzigem Leprosorium behandelt wurden, und von denen Blutproben genommen werden konnten, haben wir die Serumproteine, das Fibrinogenmenge des Plasmas und den Lipoidgehalt des Serums untersucht.

Unser Material umfasste den grössten Teil von den in Finnland befindlichen Leprafällen, 3 anesthetica (A) und 5 tuberosa (T)-Formen (Tabelle Nr. I.)

Die Blutproteine wurden elektrophoretisch nach Olhagen (8) bestimmt, und die Resultate gehen hervor aus der Tabelle Nr. II. Die Bestimmung des Gesamteiweissgehaltes des Serums wurde nach der Mikro-Kjeldahl-Methode durchgeführt (als N-Koeffizient wurde 6.25 gebraucht. Der Reststickstoff, welcher desgleichen nach Kjeldahl bestimmt wurde, ist in den Rechnungen berücksichtigt).[1] Die Fibrogenbestimmung basierte auf der Rekalzifikation des durch Zitratzusatz erhaltenen Plasmas und auf der gravimetrischen Bestimmung des Ausfalls. Sowohl die für die Totalproteine als auch für das Fibrogen mitgeteilten Werte sind Mittelwerte aus zwei Paralle-Analysen, deren Maximal-Differenz 3% bezw. 5% war.

Die Lues-Seroreaktionen und die Thymoltrübungsreaktion wurden mit Verfahren durchgeführt, welche eingehend an anderer

[1] Es dürfte angezeigt sein, hier zu bemerken, dass der Reststickstoffwert bei allen Patienten normal war (zwischen 23 u. 40 mg %).

TABELLE II

DIE BEI LEPRA ANESTHETICA UND TUBEROSA ERHALTENEN PROTEIN- U. FIBRINOGENWERTE DES BLUTES

	Nummer	Geschlecht	Tot. prot. g %	Alb.		Globuline								A/G Verhältnis	Fibrinogen g %
				rel. %	g %	α rel. %	β rel. %	γ rel. %	Tot. rel. %	α g %	β g %	γ g%	Tot. g %		
Tuberosa T	4	♂	7.71	60.2	4.65	5.1	12.5	22.2	39.8	0.39	0.96	1.74	3.06	1.5	0.96
Anesthetica A	1	♀	7.44	56.1	4.19	7.5	15.8	20.6	43.9	0.56	1.17	1.52	3.25	1.3	0.51
	5	♀	7.85	54.5	4.30	7.5	13.2	24.8	45.5	0.59	1.03	1.93	3.55	1.2	0.53
	8	♀	8.00	35.1	2.81	8.9	13.3	42.7	64.9	0.71	1.06	3.42	5.69	0.5	0.57
	2	♀	9.01	37.1	3.35	8.8	11.2	42.9	62.9	0.79	1.01	3.86	5.66	0.6	0.46
	3	♂	9.32	36.0	3.36	5.4	13.2	45.4	64.0	0.50	1.23	4.22	5.95	0.6	0.58
	7	♀	7.77	49.1	3.80	9.6	11.1	30.2	50.9	0.75	0.83	2.33	3.97	1.0	0.48
	6	♀	8.33	46.1	3.86	13.3	11.9	28.7	53.9	1.10	0.99	2.38	4.47	0.9	0.43

Stelle beschrieben worden sind (1,9). Beurteilung der Thymolreaktion; negativ (−) < 500, unsicher positiv (±) 500−600, deutlich positiv (+) > 600.

Die Totallipoide und Phosphatide sind nach Bloor (2, 3) bestimmt worden, das Cholesterin mit einer Modifikation dieses Verfahrens (14)[1]. Die Neutralfette wurden desgleichen nach Bloor berechnet, indem von den Totalfettsäuren die im Cholesterin und den Phosphatiden enthaltenen Fettsäuren abgezogen wurden.

Die Blutproben wurden morgens bei leerem Magen von den liegenden Patienten entnommen. Die Qualität des Serums bezüglich der Haemolyse wurde mit der sog. Pyramidonprobe von Ivanov kontrolliert. Da das Resultat in allen Proben negativ war, hat die Haemolyse die Ergebnisse also nicht beeinflusst.[2]

RESULTATE[3]

Die Totalproteine: In den Anesthetica (A)-*Fällen* (7 St.) sind die Werte normal, durchschnittlich 7.6, obwohl sie in zwei Fällen

[1] Siehe Diskussion (Stevenson). Möglicherweise hat die Haemolyse auch auf die Resultate anderer Autoren Einfluss ausgeübt.

[2] Für seine grosse Hilfe in Totallipoid- und Phosphatidebestimmungen möchten wir auch Herrn mag. phil. Louhivuori herzlich danken.

[3] Normalwerte nach Olhagen: Tot. prot. 6.5—8.0 Alb. 54.7—62.5 rel. % 3.85—4.8 g % α 5.3—7.9 % B 13 1—16,6 % γ 16.2—24.5 g % glob 37.5 —45.3 g % A/G Verhältnis 1.28—1.69.

TABELLE III

DIE BEI LEPRA ANESTHETICA UND TUBEROSA ERHALTENEN LIPOIDWERTE

Nummer	Lipoide				
	Total mg% Fettsäuren	Cholesterol		Phosphatide mg%	Neutral-fettsäuren mg%
		Total mg %	Vereste-runggrad		
4	469	175	36	157	341
1	701	195	38	57	638
5	633	120	53	125	515
8	650	137	54	41	587
2	—	—	—	—	—
	—	147	48		
3	771	137	54		
7	618	156	67	134	484
6	1111	195	70	210	926

ziemlich gross sind. In den Tuberosa (T)-Fällen (5) sind die Werte durchschnittlich grösser als normalerweise, nämlich 7.8—9.3, durchschnittlich 8.5. Insbesondere Nr. 2 und 3 sind gross.

Die Albumine: Bei der A-Form sind die Werte normal, bei der T-Form hingegen alle herabgesetzt, durchschnittlich ziemlich viel. Absolut sind 8, 2 und 3 erniedrigt, während wiederum 6 und 7 normal sind.

Die Globuline. ($\alpha+\beta+\gamma$) In den A-Fällen sind sie normal. Bei den T-Fällen überall kräftiger Anstieg (am wenigsten in Fall 7, am meisten in Fall 3). Ausgesprochen dieser Anstieg muss als durch die Zunahme von γ bedingt angesehen werden, welche letztere bei Nr. 3 gewaltig ist, und gerade hierauf ist zunächst auch der grosse Wert der Totalproteine, 9.3%, zurückzuführen. Die α-Globuline sind in der A-Gruppe sowohl relativ als auch absolut normal. In der T-Gruppe sind sie nur bei Nr. 6 absolut vermehrt. Die β-Globuline sind relativ normal sowohl in der A- als auch in der T-Gruppe. Absolut sind sie in der Gruppe A normal, in der T-Gruppe zeigen die Nummern 2 und 3 etwas Zunahme, während wiederum 6 und 8 normal sind. β-Anomalie fehlt in allen Fällen. Die γ-Globuline sind in der A-Gruppe sowohl relativ als auch absolut etwas erhöht. Bei den Fällen 2, 3 und 8 der T-Gruppe

TABELLE IV

DIE BEI LEPRA ANESTHETICA UND TUBEROSA ERHALTENEN WR, KAHN- U. THYMOL-RESULTATE

Nummer	Thymoltrübungseinheit	WaR		Kahn
		Gewöhnlich	Chol.	
4	fehlt	−		−
1	fehlt	−	−	−
5	357 −	−	−	−
8	641 (+) +	+	±?	+
2	909 + +	−	−	+
3	375 − −	−	−	−
7	+ 757 + +	±	+	+
8	fehlt	+?	±	+?

ist γ sowohl relativ als auch absolut beträchtlich gestiegen, in den Fällen 7 und 8 weniger, aber doch deutlich, insbesondere relativ.

Das Fibrinogen, hat in allen Fällen zugenommen und gehört in beiden Gruppen zur gleichen Grössenklasse, obschon Fall Nr. 4 aus der A-Gruppe den Mittelwert der ganzen Gruppe erhöht.

Die Lipoide. Die Totallipoide sind in beiden Gruppen beträchtlich erhöht.

Das Totalcholesterin hat in beiden Gruppen etwas abgenommen. Das Veresterungsgradprozent schwankt in der A-Gruppe 36—53 und in der T-Gruppe 48—70. Die Phosphatidwerte scheinen von den Normalwerten nur wenig abgenommen zu haben. Die Neutralfettsäuren dahingegen sind in beiden Gruppen beträchtlich gestiegen, schwankend zwischen 340 und 926 mg%.

Die Thymol-Reaktions-Werte ist vor allem in der T-Gruppe gestiegen. Der Fall 3 bildet hier deutlich eine Ausnahme. *In der T-Gruppe fällt deutliche Positivität der Seroreaktionen auf*, was Salminen (11) schon 1928 bei uns festgestellt hat.

Die drei Leprafälle, welche in Seibert und Nelsons (12) Material

über die Serumproteine im Zusammenhang mit manchen chronischen Krankheiten anthalten sind, sind unseres Wissens die *einzigen mit Elektrophorese* durchgeführten Serumanalysen (die Elektrophorese war vom Serum gemacht worden, und das Fibrinogen war auch sonst nicht bestimmt). Da das Material jedoch nur 3 Fälle umfasste, über diesen Beschaffenheit nicht anderes bekannt ist, als dass sie fortgeschrittene Falle waren, lassen sie sich für einen Vergleich in dieser Arbeit nicht anwenden. Die anderen Autoren (13, 5, 7, 4, 6, 10) haben ihre Bestimmungen mit Howe, Wu oder anderen Ausfällungsverfahren vorgenommen, so dass sie sich mit diesem Material nicht vergleichen lassen, insbesondere wenn man berücksichtigt, dass die in den referierten Untersuchungen gemachten Analysen nur das Totalglobulin betreffen, und ausserdem so weit zurückliegen, (und mit Methoden, welche sich später als sehr unzuverlässig erwiesen haben und welche insbesondere bei den pathologischen Sera ganz irreführende Resultate ergeben), dass die Analysenergebnisse mehr oder weniger unzuverlässig sind, (wie z.B. Stevenson selbst hervorgehoben hat). Die Ausführung der Lipoidanalysen und der Thymol-Reaktion sowie die Lues-Reaktionswerte ergänzen gleichzeitig unsere Untersuchung.

Es ist festgestellt worden, dass die Lipoidwerte des Totalplasmas bei der Lepra sehr niedrig sind. Auch Erniedrigung der Cholesterolwerte ist konstatiert worden. Villela, Castro und Anderson (15) dahingegen stellten hohe Totallipoidwerte fest, aber so wie auch die anderen Autoren niedrige Cholesterolwerte.

ZUSAMMENFASSUNG

Die Verfasser haben Protein-, Lipoid-, Thymol- und Lues-Seroreaktionen bei Lepra an dem ganzen in Finnland zur Verfügung stehenden Material untersucht. Das Material umfasst 8 Fälle, von welchen 3 von der Anesthetica- und 5 von der Tuberosa-Form waren.

Die Totalproteine: In den Anesthetica (A)-Fällen sind die Werte normal.

In den Tuberosa (T)-Fällen sind die Werte durchschnittlich grösser als normalerweise.

Die Albumine: Bei der A-Form sind die Werte normal, bei der T-Form hingegen alle herabgesetzt.

Die Globuline: ($\alpha+\beta+\gamma$) In den A-Fällen sind sie normal. Bei den T-Fällen überall kräftiger Anstieg.

Die α-Globuline sind in der A-Gruppe sowohl relativ als auch absolut normal. In der T-Gruppe sind sie nur bei Nr. 6 absolut vermehrt.

Die β-Globuline sind relativ normal sowohl in der A-als auch in der T-Gruppe.

Die γ-Globuline sind in der A-Gruppe sowohl relativ als auch absolut erhöht. In der T-Gruppe ist γ sowohl relativ als auch absolut beträchtlich gestiegen.

Die Totallipoide sind in beiden Gruppen beträchtlich erhöht.

Die Neutralfettsäuren dahingegen sind in beiden Gruppen beträchtlich gestiegen.

Das Thymol ist vor allem in der T-Gruppe gestiegen. In der T-Gruppe fällt deutliche Positivität der Seroreaktionen auf.

KASUISTIK

TABELLE I. DIE ZUSAMMENSETZUNG DES MATERIALS

Nummer	Geschl. u. Alter (Jahr)	Bakt. Befund (Nasenschleim)	Typus	Krankheitsdauer in Jahren	Bemerkungen
1	♀, 38	neg.	A	34	J. 1921 WR: —, Sachs-Georgi-Reaktion: —

Nr. 1. 10/4 —48.

Finger kurz, gekrümmt, sehen aus wie zweigliedrig. Haut gesund. Füsse: Auf der rechten grossen Zehe an der Basis eine querlaufende Ulzeration. Zehen kurz, IV Zehe links amputiert. Wärmeempfindung in Händen und Füssen nur teilweise.

4	♂, 59	»	»	4	Eiterung d. Ulzerationen (Unhygiene d. Patienten weil schwer schizophrenisch.)

Nr. 4. 10/4 —48.

Sitzt und murmelt für sich selbst, katatonische Bewegungen. Im allgemeinen keine sachlichen Antworten herauszubringen.

Von unterhalb der rechten Schulter auf das Schädeldach läuft ein breites, helles, haarloses, narbiges Gebiet. Finger kurz, dick, gekrümmt. Von den Nägeln nur kleine Reste. An den Fingern Brandwunden (raucht!).

5	♀, 64	»	»	47	

Nr. 5. 10/9 —48.

Hände schmal, verkümmert, klein, von den Fingern 1—1½ Glieder und Gelenke übrig, Brandwunden. An den Füssen ähnliche Veränderungen. An beiden Fusssohlen eine Ulzeration. Kann seine Zehen nicht hochheben.

Gesicht normal.

Nummer	Geschl. u. Alter (Jahr)	Bakt. Befund (Nasenschleim)	Typus	Krankheitsdauer in Jahren	Bemerkungen
2	♀, 47	pos.	T	4	

Nr. 2. 10/4 —48.

Das Gesicht und die Hände sowie die Handgelenke blaurot, knollig, geschwollen, dick. Augenwimpern und -brauen verschwunden. Finger dick, Haut unversehrt bis auf eine Brandwunde und eine gesprungene Stelle. An den Armen, Füssen, Beinen und Schenkeln flache, pigmentierte Höcker. An den Schultern und der Brust weniger. Schmerz- sowie Kalt-Warmempfindung an den Händen und Füssen mangelhaft.

3	♂, 49	»	»	20	Öfters Fieberperioden. Bruder gestorb. an Intestinal-Tuberkulose.

Nr. 3. 10/4 —48.

Rauhe Stimme. Höcker im Bezirk der Augenbrauen, an der Nase und in den Mundwinkeln rötliche Knollen, Augenbrauen verschwunden, an ihrer Stelle hängt eine Hautfalte auf das Auge herab.

Der linke Fuss vom Knöchel an einwärts luxiert, eine bis zum Knochen reichende Ulzeration an der Aussenseite. Auch der rechte Knöchel schlecht, Haut unversehrt. An den Unterarmen und den Beinen braunrote, flache Höcker und Flecken. Finger gekrümmt, kurz, dick, einige amputiert, Brandwunden. An Händen und Füssen Gefühl verschwunden.

6	♀, 56	neg.	»	3	J. 1946 WR: +? Kahn: + SR: 6 mm/St.

Nr. 6. 10/4 —48.

Im Gesicht: Am Kinn einige braunrote Ausschlagflecken von zwei mm Durchmesser. An beiden Armen, insbesondere an der Flexorseite dicht nebeneinander braunrote Flecken. Hände und Handflächen frei. Am Körper, vor allem am Rücken Flecken von 2—50 mm Durchmesser. An den unteren Extremitäten bis zur Hälfte des Unterschenkels Flecken, Füsse und Fusssohlen frei. Die Ausschlagflecken sind braunrot, undeutlich und unregelmässig begrenzt. An den Ausschlagflecken ist die Berührungs-, Schmerz- und Wärmesensibilität vermindert. Im Gesicht Haut verdickt und Furchen vertieft. Schmerzgefühl in der Narbe eines ursprünglichen Knollens im rechten Unterarm stark herabgesetzt.

Nummer	Geschl. und Alter	Bakterien befund	Typus	Krankheitsdauer in Jahren	Bemerkungen
7	♀, 58	+	»	13	

Nr. 7. 10/4 —48.

Heisere Stimme. In beiden Augen unterhalb der Cornea »Fleisch» gewachsen. Erbsengrosse, bräunliche Flecken an den Wangen und am Kinn. Finger kurz, gekrümmt, dick, verkürzt, Brandwunden. Blaurote, dicke Grenze nach »Pickeln» am Handgelenk usw. Peronaeustypische Lähmung an den Füssen. An den Füssen einige Narben.

8	♀, 72	pos.	»	11	Probe-Exzision: histol. Bild v. Lepra, Leprabakt (J. 1944 Prof. A. Saxén) J. 1944 WaR: +? Kahn: +

N:o 8. 10/4 —48.

Atembeklemmung und Herzbeschwerden in der letzten Zeit, hat Digitalis bekommen. Gesicht und Hände bläulich braun. Am Rücken und sonst am Körper braune Flecken. Flache, kleine Höcker an der Nase, den Lippen, den Wangen und im Bezirk der Augenbrauen. Augenwimpern und -brauen verschwunden. An den Händen und den Unterarmen Knollen, Haut verdickt, unversehrt. Keine eigentliche Gefühllosigkeit. Füsse wie die Hände. — Stimme etwas heiser.

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FROM THE VIRUS LABORATORY OF THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY, HELSINKI UNIVERSITY, AND THE STATE SERUM LABORATORY

STUDIES ON THE EFFECT OF SONIC TREATMENT ON HEMAGGLUTINATING VIRUSES AND ON INHIBITORS OF VIRUS HEMAGGLUTINATION

I. THE EFFECT OF SONIC TREATMENT ON INFLUENZA AND MUMPS ALLANTOIC FLUIDS, ON INHIBITORS OF VIRUS HEMAGGLUTINATION AND ON VIRUS-INHIBITOR MIXTURES

by

KARI PENTTINEN [1, 2]

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Little is known of the effect of sonic energy on viruses. Some experiments have been made with tobacco mosaic virus which indicate that the infectivity of the virus is destroyed by sonic treatment if the effect of cavitation is not prevented by removing the air from the fluids. (7, 10, 13, 14). The experiments with animal viruses and sonic vibration are scanty. Scherp *et al.* (12) have found that the infectivity of the influenza virus was not easily destroyed by sonic energy. Wiener *et al.* (15) released by intense sonic vibration the 30S antigen from preparations of the 600S component of the influenza virus. Hopwood *et al.* (4, 5) state that the titer of vaccinia lymph increases during sonic treatment. Rivers *et al.* (11) could not confirm this result, but observed that the infectivity titer of vaccinia virus preparations did not decrease if the virus was protected by other proteins. Similar result were obtained by Yaoi *et al* (16). Scherp *et al.* (12) could not destroy the infectivity of poliomyelitis

[1] Aided by a grant from Sigrid Juselius Foundation.
[2] With the technical assistance of Mrs. Pirkko Koivunen.

virus by sonic treatment. Kasahara *et al.* (8) found that Japanese and St. Louis encephalitis viruses are rapidly rendered non infectious by sonic oscillations of high frequency. Some experiments with bacteriofages indicate that they are fairly sensitive to sonic energy. (1, 9).

The conflicting reports in the literature are very probably due to different conditions in experiments with sonic energy.

When investigating the effect of sonic treatment on hemagglutinating viruses, some observations were made suggesting that sonic treatment of infected allantoic fluids increased the hemagglutinating titer of preparations. Other preliminary experiments showed that sonic energy had an destructive effect on the egg white inhibitor of virus hemagglutination. This paper describes experiments which confirm the above-mentioned results and which possibly give an explanation on the increase in hemagglutinating titer during sonic treatment.

THE EFFECT OF SONIC ENERGY ON HEMAGGLUTINATING TITER AND INFECTIVITY OF INFLUENZA AND MUMPS ALLANTOIC FLUIDS

Method. — Embryonated eggs incubated for 7 or 10—11 days at 37.5° C—38.0° C were infected into the allantoic sac with 10^{-3} dilution of mumps or influenza virus allantoic fluids respectively. The diluent contained 0.2 per cent bovine albumin (Poviet Producten N. V. Amsterdam) and 0.067 M phosphate buffer of *p*H 7.2. The mumps virus strain was the Enders strain, which was passed in our laboratory through 18 eggs intra-allantoically before the experiments in question. The influenza strains were PR8 198—M593—E107 and Lee F8—M139—E153. The eggs infected with influenza strains were incubated at +36°C—36.5°C for 48 hours, and refrigerated overnight at +4° C. The following morning the allantoic fluids were collected and centrifuged at 1500 r.p.m. for 10 minutes. The experiments with allantoic fluids were usually carried out on the same day or occasionally two or three days later. The mumps allantoic fluids were prepared similarly with the exception that incubation at + 36.0° C— + 36.5° C was continued for 5 days.

The sonic treatment of infected allantoic fluids was carried out with the Raytheon 9KC 50W Magnetostriction Oscillator Model S—102A. The frequency control was, according to the instructions, kept at the optimal position and the output voltage meter at 150 V.The alternating current in our laboratory has 50 cycles instead of 60, for which the oscillator has been planned.

10 ml of infected allantoic fluids were put into the oscillator cup; the running time was up to 20 minutes. Specimens of 0.2 ml were taken

at suitable intervals. The cup of the oscillator was sterilised by ultraviolet light before use.

The hemagglutination titrations of allantoic fluid were carried out according to the pattern method in duplicate series, the one starting from the final dilution of 1/40 and the other from 1/56.4. 0.5 ml of 0.5 per cent chicken cells were added to the allantoic fluid dilutions and readings were made after 45—60 minutes. In the infectivity titrations the allantoic fluids were diluted in multiples of ten in the above mentioned buffered albumine solution. From 5 to 7 eggs were used for each dilution. The hemagglutinating effect of the allantoic fluid was considered as prove for the presence of virus.

In Table 1 are presented the results of hemagglutination titrations of PR8, Lee and mumps allantoic fluids before and after sonic treatment.

TABLE 1

THE EFFECT OF SONIC TREATMENT ON THE HEMAGGLUTINATING TITER OF PR8, LEE AND MUMPS-VIRUS ALLANTOIC FLUIDS

Time of Treatment	Hemagglutinating titers.[1]			
	PR8	Lee	Mumps Virus	Uninfected Allantoic Fluid
0 min	3.26	2.95	2.35	<0.3
5 min	—	—	3.26	—
10 min	—	3.26	3.26	—
20 min	3.41	3.26	3.26	<0.3

[1] The titers are expressed as the logarithms of the reciprocals of titers.

The rise in hemagglutinating titer during sonic treatment cannot be demonstrated in all infected allantoic fluids. The results presented in Table 2 can, however, be considered as typical when infected allantoic fluid pools are tested. As can be seen, the effect is most marked with mumps virus allantoic fluid pools, but also the PR8 and Lee allantoic fluid pools show the same tendency in varying degrees.

The results of the infectivity titrations with the infected PR8, Lee and mumps allantoic fluids, which show an increase in hemagglutinating titer during sonic treatment, do not show any increase in infectivity titer. The infectivity titers of PR8 and Lee fluids do not decrease during 20 minutes treatment with sonic oscillator. This is in good agreement with the results of Scherp *et al.* (11).

The results with the mumps virus allantoic fluids are not completed due to the difficulties to obtain sharp endpoints in infectivity titrations with the virus.

As an explanation of the increase in hemagglutinin titer it can be assumed that sonic vibrations simply disrupt virus aggregates. The other possibility is that sonic treatment disintegrates inhibitors of virus hemagglutination and releases viruses which are bound to inhibitors and inactive in hemagglutination tests. It is also possible that sonic vibrations disrupt viruses to smaller units, which still have hemagglutinating properties. According to Hoyle (6) it is possible to increase the hemagglutinin titer of influenza virus preparations by ether treatment, which, according to him, disrupts the lipoid wall of virus. The small changes in infectivity titers, however, speak against the assumption of disruption of viruses. Because the disruption of virus aggregates might also be connected with the disintegration of inhibitors, which might bind viruses to aggregates, the part played by inhibitors has been investigated in the following experiments.

THE EFFECT OF SONIC ENERGY ON ALLANTOIC FLUID AND EGG WHITE INHIBITORS OF VIRUS HEMAGGLUTINATION

Method. — Unpurified allantoic fluid and egg white preparations were used. The allantoic fluid was collected from eggs incubated for 14 days and centrifuged at 1500 r.p.m. for 10 minutes. The experiments were carried out before any precipitation of allantoic fluid had occurred. Egg white preparations were made pooling the egg white from several eggs, filtering the pools through gauze and centrifuging the fluid at 1500 r.p.m. for 20 minutes. Allantoic fluid was undiluted treated with sonic energy and egg white in a dilution of 1/10, using phosphate buffer as diluent. The inhibition titer of the preparations was determined as follows: The dilutions were made in 0.25 ml volume, with 2 as the dilution factor. 0.25 ml of heated Lee allantoic fluid dilution was then pipetted into each tube. The dilution of Lee allantoic fluid used was such, that in the final 1.0 ml volume were four agglutinating units of the virus. The virus-inhibitor mixture was then incubated at room temperature for 30 minutes, after which 0.5 ml of 0.5 per cent chicken cells were added. The readings were made after 45—60 minutes at room temperature. The Lee allantoic fluid used as indicator of the inhibitory activity of the preparations was heated for 30 minutes at 56° C. In each series a control titration of the used Lee allantoic fluid dilution was included to make sure that the virus content in the test was four agglutinating units.

TABLE 2

THE EFFECT OF SONIC TREATMENT ON THE PROPERTY OF ALLANTOIC FLUID AND EGG WHITE TO INHIBIT VIRUS HEMAGGLUTINATION

Time of Treatment	Inhibition Titer[1]	
	All. Fluid	Egg White
0 min	1.51	3.71
1 min	1.20	2.81
3 min	1.20	2.51
5 min	1.20	2.20
10 min	0.90	1.90
20 min	0.90	1.90

[1] The titers are expressed as the logarithms of the reciprocals of titers.

In Table 3 are presented the results of two experiments where allantoic fluid and egg white were treated with sonic energy, as mentioned earlier. The inhibitory activity of the preparations was determined after treatment for 1, 3, 5, 10 and 20 minutes.

As can be seen from Table 3, the sonic treatment decreases the titer of allantoic fluid and whole egg white to inhibit virus hemagglutination. The egg white is very sensitive, and in the beginning of the treatment, in the conditions in question, the fall in the inhibiting titer is very rapid. Flosdorf and Chambers (3) have shown that sound vibrations also denaturate egg albumin and change its antigenic properties. Allantoic fluid is less sensitive to sonic treatment, and accordingly the time of treatment has to be longer in order to get a clear fall in titer.

The destructive effect of sonic treatment on inhibitors of virus hemagglutination can be shown also with PR8 strain and mumps virus as indicators. Infected allantoic fluids either untreated or inactivated with heat can be used. With untreated fluids as indicators the changes in inhibiting titers are small but reproducible. The most suitable temperature for heat inactivation was with PR8 allantoic fluid 61° C and with mumps allantoic fluid 48° C when the inactivation time was half an hour.

When testing the effect of *p*H on the destruction of the inhibitory activity of egg white by sonic treatment it was observed that no remarkable differences existed between *p*H values 6.2 and 8.2, the effect of the treatment beeing possibly a little more marked at *p*H 7.0.

THE EFFECT OF SONIC ENERGY ON VIRUS-INHIBITOR MIXTURES

Method. — Equal amounts of normal allantoic fluid and virus allantoic fluids were mixed and incubated for 30 minutes at room temperature. 10 ml of the mixture was then treated with sonic energy as mentioned before. As a control a mixture of phosphate buffer and infected allantoic fluid was treated in the same way. The technique with egg white was the same except that a dilution of 1/10 was made in phosphate buffer before the experiment. The hemagglutinating titer of the mixtures was determined before and during sonic treatment, as mentioned before.

In Tables 4 and 5 are presented the results of the experiments where heat-inactivated Lee allantoic fluids were mixed with normal allantoic fluid and eggwhite dilution, incubated for 30 minutes, and the mixtures treated with sonic energy for 10 minutes.

TABLE 3

THE EFFECT OF SONIC TREATMENT ON A MIXTURE OF HEAT-INACTIVATED LEE ALLANTOIC FLUID AND NORMAL ALLANTOIC FLUID

Time of Treatment	Hemagglutinating Titers [1]		
	Lee + Normal All. Fluid [2]	Lee + Phosphate Buffer [2]	Normal All. Fluid
0 min	1.60	2.95	<0.3
5 min	2.95	—	
10 min	2.95	3.11	<0.3

[1] The titers are calculated to unmixed allantoic fluid and expressed as the logarithms of the reciprocals of titers.
[2] Incubated at room temperature for half an hour.

TABLE 4

THE EFFECT OF SONIC TREATMENT ON A MIXTURE OF HEAT-INACTIVATED LEE ALLANTOIC FLUID AND EGG WHITE DILUTION

Time of Treatment	Hemagglutinating Titers [1]		
	Lee + Egg White Dilut. [2]	Lee + Phosph. Buffer [2]	Egg White Dilution
0 min	1.75	3.41	<0.3
5 min	2.81	—	—
10 min	3.11	3.41	<0.3

[1] The titers are calculated to unmixed allantoic fluid and expressed as the logarithms of the reciprocals of titers. The Lee allantoic fluid pool used in the experiments of Table 5 was not the same, which was used in Table 4.
[2] Incubated at room temperature for half an hour.

As can be seen from Tables 4 and 5, a sonic treatment of 10 minutes, in the conditions in question, increases the titer of hemagglutination inhibitor and virus allantoic fluid mixtures to nearly the titer of the control mixtures. The same type of phenomenon can be reproduced also with PR8 and mumps virus allantoic fluids, especially with heat-inactivated fluids. When working with noninactivated PR8 and mumps virus fluids the rise in titer is much less because the effect of the inhibitor preparations to unheated virus allantoic fluids is small (2).

The experiments with heat-inactivated virus and inhibitor mixtures suggests that the rise in hemagglutinating titer of infected allantoic fluids during sonic treatment might be due to deliberation of inactive virus from inhibitors.

It is, however, uncertain whether the increase in hemagglutinating titer is due solely to destruction of inhibitors, the allantoic fluid inhibitor beeing relatively resistant to sonic treatment as Table 2 shows.

It seems, that sonic treatment might be a method for measuring how much allantoic fluids contain inactive virus bound to inhibitors of hemagglutination.

SUMMARY

The writer has investigated the effect of sonic energy on influenza A (PR8) and B (Lee) and mumps virus (Enders strain) allantoic fluid pools. Experiments were also made to investigate the effect of sonic treatment on allantoic fluid and egg white inhibitors of virus hemagglutination and on virus-inhibitor mixtures. The results were:

1. Sonic treatment increases the hemagglutinating titer of virus preparations in varying degrees.
2. Sonic treatment, in the conditions in question, does not easily affect the infectivity of the virus strains investigated.
3. Sonic treatment decreases the hemagglutination-inhibiting titer of egg white, as well as that of allantoic fluid. The former is more sensitive than the latter.
4. Sonic treatment increases the hemagglutinating titer of inhibitor-virus mixtures to nearly the titer of buffer-virus mixtures.

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FROM THE PARASITOLOGICAL DEPARTMENT OF THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY, UNIVERSITY OF HELSINKI

OBSERVATIONS ON THE SYMBIOSIS OF SOME FUNGI AND BACTERIA

By

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The symbiosis of the fungi and the bacteria has been treated but slightly in the literature (1). Hansen *et al.* (2) claim that the lactobacillus, when growing alone in the carbohydrate nutrient medium or in saliva, does not unfold low degrees of *p*H nearly as fast as when in the symbiosis with the yeast fungus. Stephan and Hemmens (3) came to the conclusion that the acidity, reached in the symbiosis generally remains between those values of *p*H which are generated by both components separately. In their experiments the time of observation, however, was limited to 0—150 minutes, and in the course of this time they discovered that the micro-organisms are able to consume, at least partly, the amount of acid they have developed. In other words, in these experiments the most marked fall of *p*H takes place immediately at the beginning of the experiment and changes only slowly thereafter.

There might be a question of symbiosis between the bacteria and the fungi in clinical cases of mixed infection often observed. Catanei (4) found out that the staphylococcus considerably stimulated the growth of the fungus favus, even in dead strains. Pätiälä (5) again, discovered that certain dermatophytes have an antibiotic effect *in vitro* at least against *Staphylococcus aureus*. According to Thoma (6), the most important bacterium in the cases of caries pulpitis ostitis would be *Streptococcus viridans* which is often found together with the actinomyces. Korttila (7) has taken specimens of

perforated and unperforated ulcers during the operation and found a large strain of streptococci together with yeast fungi in most cases. Hayes (8) states that large amounts of *Staphylococcus albus* stimulate the growth of *Streptococcus viridans*, while large amounts of *Staphylococcus aureus* prevent it. He presents a hypothesis that *Staphylococcus aureus* might generate some substance which would have a toxic effect on the growth of *Streptococcus viridans*.

The mouth is the natural environment where the bacteria and the fungi can well grow together. In spite of clinical pictures which are known in connection with the thrush and actinomycosis, there are almost regularly to be found in the mouth fungi which can be regarded as saprophytes. The writer has discovered in statistical investigations made at the Departments of Odontology and Serology and Bacteriology of Helsinki University (figures not yet complete) that yeast fungi are to be found mostly in neglected months, fairly often in well cared for and least of all in toothless mouths. Since there are always also bacteria in the mouth where especially lactobacilli, strepto- and staphylococci have been considered important factors in the etiology of the caries process, it is very interesting to see whether there is a phenomenon similar to the symbiosis between these two micro-organic groups.

OWN INVESTIGATIONS

The following aspects have been taken as central problems in the experiments:

1. Do the bacteria remain longer viable in the nutrient medium together with some fungus than alone?
2. Are the bacteria viable with the fungus in the lower pH rather than alone?
3. Do the bacteria change the degree of acidity of the nutrient medium more when growing there together with the fungus than alone?

The micro-organisms of the experimental series are as follows:

— *Candida albicans* (»Kirurgi Koskela»), Syn. Monilia alb., Oidium alb.

— *Candida tropicalis*.

— *Torulopsis utilis* (»B III 2, Microbiological Institute»).

— *Streptococcus haemolyticus β* (»S. 84»).

— *Staphylococcus aureus* (»T.K.»).
— *Staphylococcus albus* (»93961»).
— *Lactobacillus acidophilus* (»Packalen»).
— *Escherichia coli* (»3203 Mustakallio»).

As the names indicate, the micro-organisms have not been taken directly from their natural surroundings but are old laboratory strains.

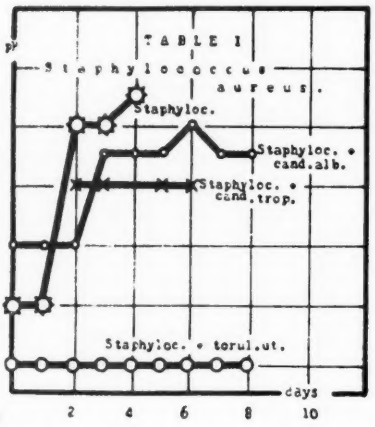
In the preliminary experiments, the suitable dilutions were determined in view of a sufficiently strong culture which did not, however, cover the growth of the other micro-organism. Raulin's solution was used as nutrient medium. The acidity of this solution has been standardized as *p*H 5. When adding HCL and NaOH to this solution, the following *p*H series were received: 3—4—5—5.5—6—6.5—7.5—5—8—9. The degree of acidity was determined colorimetrically. In this solution it was found suitable to cultivate first the fungi alone for five days at 37° C; after this *p*H was determined once more, and then the bacteria were planted into the test tubes in order to be investigated for symbiosis. The 0-control was performed in the usual way. Observations were made daily for eight days. For this a specimen taken from Raulin's tubes was cultivated on a serum and blood-agar plate, of which the reading was made at 37° C after one day's growth. A Gram staining was made of every reading for control purposes.

I have presented the results in the following by graphic tables made separately for each bacterium. In the same co-ordination we see the growth of the bacterium alone and together with the fungus which was present in the experimental series.

The lowest value of *p*H has been taken as the variable of the set of graphs where the growth of the bacterium is positive with certainty. As regards the fungi, the corresponding value has not been presented at all, because it was strongly positive in the whole scale of *p*H used. The acidity after the symbiosis of the bacteria and *Candida tropicalis* could not be determined owing to an obstacle present, and therefore the comparison between this value and the change of *p*H caused by the fungus alone cannot be made. The table of numbers left of the co-ordination presents in columns the final values of *p*H in various combinations, the starting value being nearest to the graphs.

pH after 5 days (Torul. ut.)	pH at the end (Staphyloc. + Torul. ut.)	pH after 5 days (Cand. trop.)	pH at the end (Staphyloc. + Cand.trop.)	pH after 5 days (Cand.alb.)	pH at the end (Staphyloc. + Cand.alb.)	pH at the end (Staphyloc.)	pH in the beginning of the experiment
3,5	4	7,5	7	7,5	7	9	
3,5	4	7	7	6,5	7,5	8	
>4	3,5	7	6,5	6,5	>7	7,5	
4	4	7	>7	7	7,5	7	
4	4	6,5	6	6,5	7	6,5	
4	3,5	7	>5	5,5	7	6	
3,5	3,5	6	4	>6	6	5,5	
3	6	6	<5	3	7	5	
>2	2	4	2,5	2	4,5	4	
2	1,5	3	2	1,5	2	3	

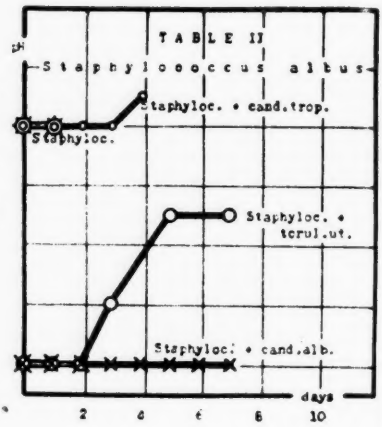
TABLE I



1. *Staphylococcus aureus.* — *Staphylococcus aureus*, when growing alone, dies in Raulin's solution after a period of four days and remains viable with *Candida albicans* until the end of the experiment (eight days), with *Torulopsis utilis* eight days, and with *Candida tropicalis* six days. The bacterium is viable with *Candida albicans* in *p*H 3—5, with Torulopsis utilis in *p*H 2—3, with *Candida tropicalis* in *p*H 6, but when alone not under *p*H 4. The changes in *p*H at the end of the experiment do not clearly differ from those caused by the fungus alone.

2. *Staphylococcus albus.* — The bacterium grows alone in the nutrient medium only for one day, with *Candida albicans* and *Torulopsis utilis* for seven days, and with *Candida tropicalis* for four days. When comparing the degrees of acidity of the nutrient medium one notices that the staphylococcus does not grow under *p*H 7 alone or with *Candida tropicalis*. With *Candida albicans* it is able to grow in *p*H 1.5—3, with *Torulopsis utilis* in *p*H 1—3. The

pH after 5 days (Torul. ut.)	pH at the end (Staphyloc. + Torul.ut.)	pH after 5 days (Cand. trop.)	pH at the end (Staphyloc. + Cand.trop.)	pH after 5 days (Cand.alb.)	pH at the end (Staphyloc. + Cand.alb.)	pH at the end (Staphyloc.)	pH in the beginning of the experiment
3,5	4	7,5	7	7	8	9	
3,5	4	7	7	7	7,5	8	
>4	4	7	6,5	6,5	7,5	7,5	
4	4	7	<7	6	7	7	
4	3,5	6,5	6	6	6,5	6,5	
4	3	7	<5	5	6,5	6	
3,5	3	6	4	4	6,5	5,5	
3	3	6	<5	5	4	5	
>6	3	4	2,5	2	4	4	
2	1	3	2	1,5	1,5	3	



formation of acidity deviating from the falling of *p*H caused by the fungi is not discernable after eight days.

3. *Lactobacillus acidophilus.* — The bacterium grows alone and with the fungi during the whole experiment (eight days). When the lactobacillus grows alone the lowest *p*H is 5 which, however, at the end of the experiment has gone up to *p*H 7 where the growth also takes place. With *Candida albicans* and *Candida tropicalis* the lowest *p*H is the whole time 5—7, with *Torulopsis utilis* it is capable of growing for six days in 1.5—3, while this lowest degree of acidity suddenly rises to 4—6.5. The formation of acid does not differ from that caused by the fungi alone after a period of eight days.

4. *Escherichia coli.* — The bacterium remains viable when growing alone during the course of the whole experimental series as well as together with *Candida albicans.* With *Candida tropicalis* it dies after six days and with *Torulopsis utilis* after five days. When alone it does not grow under *p*H 5, but keeps alive with

pH after 5 days (Torul. ut.)	pH at the end (Lactob. + Torul. ut.)	pH after 5 days (Cand. trop.)	pH at the end (Lactob + Cand. trop.)	pH after 5 days (Cand. alb.)	pH at the end (Lactob. + Cand. alb.)	pH at the end (Lactob.)	pH in the beginning of the experiment
3,5	4	7,5	7	7,5	7,5	9	
3,5	4	7	7	>8	>8	8	
<4	4	7	6,5	7	7	7,5	
4	4	7	<7	7,5	7,5	7	
4	3	6,5	6	7	7	6,5	
4	3	7	5	>5	>5	6	
3,5	2,5	6	4	4	>4	5,5	
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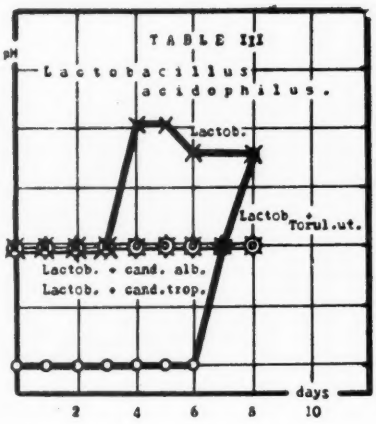
>2	2,5	4	2,5	2	2	4	
2	1,5	3	2	<2	<2	3	



Candida albicans in *p*H 1.5—3, with *Candida tropicalis* in *p*H 3 and with *Torulopsis utilis* in *p*H 1.5—3. After eight days no formation of acid could be perceived.

5. *Streptococcus haemolyticus β.* — In spite of different bacterial dilutions used in the experiment is was not possible to keep the streptococcus viable alone or together with the fungi in Raulin's solution.

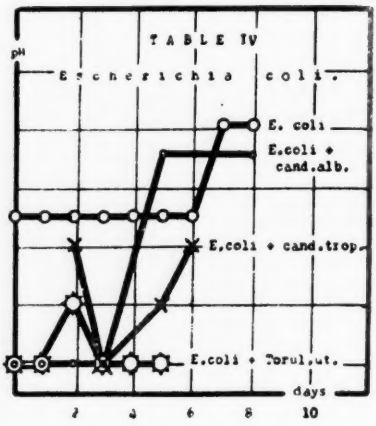
CONCLUSION AND SUMMARY

When growing in Raulin's solution, in *p*H series 3—4—5—5.5—6—6.5—7—7.5—8—9, *Staphylococcus aureus* and *Staphylococcus albus* prove to remain longer viable together with the yeast fungus than when alone. The same phenomenon is perceived also in *Escherichia coli* and in *Lactobacillus acidophilus*, but only in the lower values of *p*H.

All bacteria in the experiment except the streptococcus are able

pH after 5 days (Torul. ut.)	pH at the end (E.coli + Torul. ut.)	pH after 5 days (Cand.trop.)	pH at the end (E.coli + Cand.trop.)	pH after 5 days (Cand. alb.)	pH at the end (E.coli + and.alb.)	pH at the end (E.coli)	pH in the beginning of the experiment
3,5	4	7,5		7	10	10	9
3,5	4	7		7	10	10	8
<4	4	7		6,5	10	9	7,5
4	4	7		<7	10	9	7
4	4	6,5		6	9	6	6,5
4	3,5	7		<5	5	6	6
3,5	3,5	6		4	4	5	5,5
3	3	4		<5	5	5	5
>2	2	4		2,5	2	4	4
2	1,5	3		2	1,5	2	3

TABLE IV



together with yeast fungi, to remain viable in a much more acid solution than when growing alone.

The change of *p*H during the experiment was probably caused principally by fungi.

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FROM THE DEPARTMENT OF PATHOLOGIC ANATOMY, UNIVERSITY OF HELSINKI

REVERSIBILITY OF METAPLASIA IN THE BRONCHIAL EPITHELIUM

OBSERVATIONS ON WHITE RATS TREATED WITH COAL TAR

by

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Experimental studies have shown that the transformation of cylindric epithelium into squamous epithelium, i.e. epithelial metaplasia, is, under certain circumstances, a reversible phenomenon. In several experimental animals, for instance the rat, a metaplastic epithelium developing e.g. in the bronchi under deficiency in vitamin A, can be replaced by cylindric epithelium after the administration of vitamin A (4).

Metaplasias produced by oestrogenic hormones and their synthetic substitutes in the prostate of the test animals are also reversible (1).

In my earlier studies (3) I endeavoured to show that there is a tendency towards healing of the bronchial metaplasias, seen in various inflammatory lesions both in man and experimental animals. However, this could not be conclusively proved in these earlier series. It is true that some human cases showed transformed epithelium, whose surface parts were formed from squamous epithelium in an advanced state of differentiation from the morphological point of view, whereas the basal cellular strata consisted of cylindric cells. It is conceivable that this was a gradually progressing reparative phenomenon.

I feel that the observations to be reported here, made in the present series, furnish conclusive proof that bronchial metaplasias produced by inflammation are reversible changes.

MATERIAL

The series consisted of 15 white rats which were approximately 1 year old at the beginning of the experiment. Each animal was at first injected with 0.1—0.2 ccm 50 per cent coal tar, introduced intratracheally in olive oil. Thereafter their skin was brushed with undiluted tar once or twice a week over a period of 2 months, provided the animals survived that long. The diet recurred to has been described by me in my earlier publication (3), and experience has shown that it contained fully adequate amounts of vitamin A.

GENERAL FINDINGS

In the animals who died immediately following the injection, pronounced epithelial desquamation could be seen in the trachea and the bronchi. In 1—2 weeks strong epithelial regeneration could already be found. At first the proliferative epithelium consisted of indifferent cells. At a latter stage, in animals who survived for more than 2 months, it was possible to demonstrate stratifield epithelium with clear metaplastic changes. The cells were large, rich in protoplasm, pale, with strongly staining nucleoli. Intercellular bridges were clearly visible. In places with the highest differentiation the surface cells were desquamating non-nucleated squamac, i.e. non-cornified squamous epithelium.

Morphologically the metaplastic regenerative process was similar to that found in avitaminosis A, or as in the rats treated with 1:2:5:6-dibenzanthracene previously described by me.

Strong inflammation developed in the tracheal and bronchial walls, with fibrotic changes in the later stages. The greater tendency of the tracheal wall towards healing was evident, as previously found by me in regard to dibenzanthracene as well.

SPECIAL FINDINGS

Two animals which survived for 4 and 5 months respectively were suitable subjects for observations on metaplastic reversibility in several different regions of the trachea, and also in the right main bronchus of one of the animals.

The appended photomicrograph (Fig. 1) shows one such place in the tracheal wall. It reveals, on the surface of cylindric epithelium, some layers of cells forming an epithelium with clear intracellular bridges. Degenerative changes are present in the cells of this des-

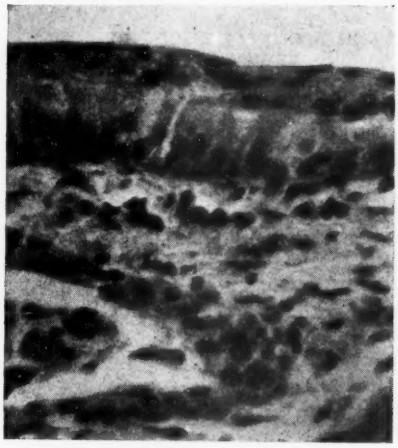

Fig. 1. — Desquamating metaplastic epithelium on the surface of cylindric epithelium.

quamating epithelium. They show shrinkage, their nuclei are pyknotic or entirely absent.

If the marginal region of such an area is studied, it becomes visible that the replacement of such metaplastic epithelium by cylindric epithelium is gradual. As the first sign of improvement of metaplasia, cuboidal or low cylindric cells appear above the basal cellular layer instead of polygonal cells. They are often more or less transversal in regard to their base. According to the growth of the longitudinal axis of the cells, they assume a perpendicular position.

The more pronounced the morphologic differentiation in the ciliated cells, the sharper the line of demarcation between the desquamating squamous epithelium and the cylindric epithelium in the process of development.

The subepithelial connective tissue in the areas described above showed slight or moderate fibrotic changes and oedema. Inflammatory cells were chiefly mononuclear, and they were relatively scarce as compared to the preceding stages.

DISCUSSION

This paper describes how a metaplastic process of regeneration developed in the tracheal and bronchial epithelium of rats treated with coal tar. In places with the highest differentiation, non-cornifying squamous epithelium was seen.

It could be demonstrated on two animals which survived for 2 and 3 months respectively after the close of the experiment, that metaplastic squamous epithelium was replaced by the cylindric variety. One can assume that the cause of this reparative process is the subsidence of inflammation, which favours the manifestation of a potency in the basal cells corresponding to that of normal conditions.

Morphologically the metaplastic regenerative process was similar with that found in rats maintained on a diet deficient in vitamin A. Likewise, the reparative phenomena were similar to those described e.g. by Wohlbach and Howe after the administration of this vitamin.

In a previous study I reported an experiment in the course of which metaplasias produced by 1:2:5:6-dibenzanthracene led to the development of pulmonary carcinoma. This process took 9 months or more. Möller (2) induced squamous cell carinoma in the lungs of rats by brushing their skin with coal tar. This als needed at least 9 months. The reparative phenomena which occurred in the course of the much shorter experiment reported here are of interest in the light of the investigations just mentioned. It is in fact generally recognised that the epithelial changes antedating experimentally induced skin carcinoma are reversible up to a certain degree. This als appears to have been the state of things in regard to changes antedating carcinoma of the lung.

SUMMARY

Rats treated with coal tar revealed metaplasia of the bronchial epithelium which was morphologically similar to that found in experimental animals maintained on a diet deficient in vitamin A. When the inflammatory process subsided, it was possible to establish a reparative phenomenon corresponding to that exhibited by these latter animals following administrations of vitamin A.

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FROM THE DEPARTMENT OF PATHOLOGIC ANATOMY, UNIVERSITY OF HELSINKI

HEALING OF PULMONARY ABSCESSES BY EPITHELISATION

EXPERIMENTAL CONTRIBUTION TO THE GENESIS OF BRONCHIECTASIS

by

K. O. NISKANEN

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It is generally recognised that chronic inflammation of the lungs is very common among laboratory rats, particularly older animals. It is also known that in this connection there is a frequent appearance of extensive purulent pulmonary cavities, which are generally interpreted as bronchiectasis. For an appreciable time, attention has been directed to bronchial metaplasia in bronchopneumonia of the rat.

Moise and Smith (4) considered as primary cause of bronchiectasis the occlusion of the bronchus by mucus, with ensuing infection. Nelson (5) reached the conclusion that the disease producing a development of bronchiectasis was due to endemic pneumonia caused by a specific virus. Cruikschank (2) advanced the opinion that the primary change was the swelling of the lymphoid tissue in the bronchial mucous membrane, possibly produced by a specific virus, and leading to bronchial obstruction. Accordingly, the accumulation and infection of the mucus are the result of bronchial occlusion.

Rats in whom vitamin A deficiency is induced also frequently develop numerous extensive pulmonary cavities, often lined with metaplastic squamous epithelium. These cavities have generally been interpreted as bronchiectasis, and its primary cause was

ascribed to bronchial occlusion produced by desquamating cornifying squamous epithelium (3, 11).

The following exposition purports to show that epithelised pulmonary cavities can develop in such a way, that abscesses of the lung are healed by epithelisation proceeding from the bronchi.

MATERIAL

The observations were made on a series comprising 18 white rats. The animals were approximately 1 year old at the beginning of the experiment. 15 animals were subjected once to an intratracheal injection of 50 per cent coal tar in olive oil, and thereafter their skin was brushed 1 to 2 times a week with undiluted tar in the course of the two first months. Three animals were allowed to inhale pitch dust 3 times a week, for one hour at a time. The animals survided from 1 day to 6 months.

OBSERVATIONS

Bronchopneumonia was a constant finding among these experimental animals. The rats treated with coal tar exhibited the main changes in the right lower and central lobe (the intratracheal injection was performed with the animal lying on its right side).

In four animals (three belonging to the former and one to the latter group) partly or wholly epithelised cavities were found in the lungs. These animals survived for 2 ½ to 6 months. There were as many as seven cavities on the same cut surface. The diameters varied between 4 and 12 mm. The largest of them comprised about half of the left lower lobe. The cavities contained suppurating, disintegrating pulmonary tissue, or were empty.

The wall of the cavity was formed in places from thick fibrous connective tissue. Howevere, fibrous changes were scarce in several places, and a clear alveolar structure was then visible. The inference is that the wall in that case was obviously formed from compressed pulmonary parenchyma. The alveolar structure was particularly clear in some trabeculae projecting into the cavity.

The character of the epithelium lining the cavities was variable. In some places normal or hyperplastic cylindric epithelium was seen. It was found especially in areas where the walls exhibited

scarce fibrotic changes, howevere, it appeared occasionally in relatively fibrotic places.

Epithelium formed from low cuboidal cells was a general occurrence. Furthermore, stratified epithelium was seen, composed in places of indifferent cells. Metaplastic epithelium with clearly visible intercellular bridges was an exceedingly common finding. The highest degree of differentiation was represented by non-cornifying squamous epithelium, whose surface cells were shedding, non-nucleated scales. Squamous epithelium was only found in walls with fibrotic changes.

Cavities whose walls are composed of pulmonary parenchyma can develop in two different ways. In the first place, an obstructing bronchitis resulting from intratracheal irritation can become the point of departure of a suppurative, ulcerating process, involving the surrounding pulmonary tissue. There are certain facts speaking on behalf of this possibility. Coal tar injected intratracheally immediately produced strong epithelial desquamation, as well as inflammation of the bronchial walls. The pneumonic process was frequently particularly pronounced in the peribronchial tissue. In the later stages, it could be found in some partly epithelised cavities that the inflammatory process continued diffusely from non-epithelised areas into the surrounding tissues. Such ulcerating regions showed close to their border-line epithelium formed from indifferent flattened cells. It is evident that with the clearing up of inflammation, epithelium can grow over the point of ulceration.

On the other hand it was possible to conclude that part of the epithelised cavities were generated in such a way hat the bronchopneumonic abscess had become epithelised from the bronchi on the line of demarcation. At first, numerous non-epithelised abscesses or carnified areas were seen in the pulmonary parenchyma. Later on, the line of demarcation of some abscesses showed bronchi with walls that were partly ulcerated. Whereas in the preserved part of the wall the epithelium was strongly proliferative, often metaplastically altered (Fig. 1). It is obvious that these bronchi are the starting point of separate areas of epithelisation which could be demonstrated in several cavities. The marginal area of the epithelial nests consisted of flattened, indifferent cells. (Fig. 2). The epithelium was spreading in this manner.

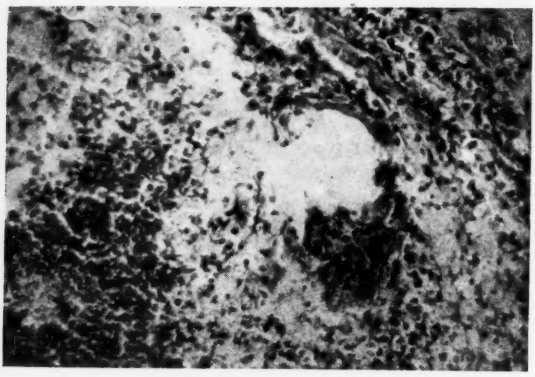

Fig. 1. — An ulcerated bronchus with proliferating transitional epithelium in the periphery of pulmonary abscess.

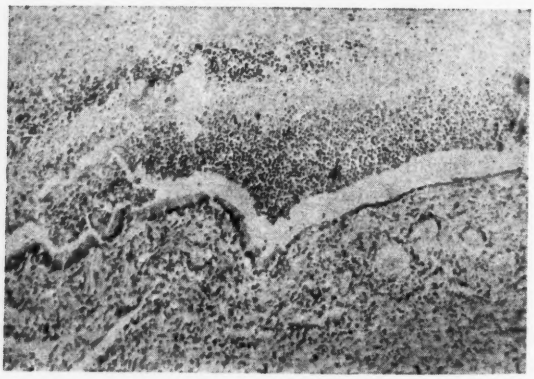

Fig. 2. — Cylindric and flat, indifferent epithelium covering the wall of pulmonary abscess.

DISCUSSION

What had been said in the foregoing is an attempt to demonstrate that the epithelised cavities appearing in the lungs can be primarily abscesses of the lung healed by epithelisation proceeding from the bronchi opened into them by the suppurative process. The epithelium lining the cavities can be indifferent, cuboidal, cylindric or squamous, evidently depending on the conditions under which the healing proceeds. Metaplasia was common in association with pronounced fibrotic changes, as the writer was also able to demonstrate in an earlier investigation of his (6).

The pulmonary changes reported here are of interest in so far as the literature contains numerous references to morphologically similar cyst-like changes in the lungs which must be interpreted as developmental disorders in the bronchi. The absence in the bronchial walls of normal elements (cartilage, musculature, elastic tissue etc.) or the irregular arrangement in the walls of the cavities have been described as phenomena characteristic of such developmental disorders. The indifferent or metaplastic epithelium lining the cysts has also been interpreted as a manifestation of the same phenomenon. Moreover, there are frequent reports on cases of carcinoma of the lung in which such congenital cystic changes have been incriminated as the starting point of the tumour (8, 9, 12, 13).

On the other hand, some recent publications (1, 7) have described similar epithelised pulmonary cavities found in man, which the authors regarded as healed bronchopneumonic abscesses. It has been feasible to follow radiologically the development of such cavities in originally normal pulmonary areas, which exciudes the possibility of congenital changes.

The foregoing as well as my own observations lend support to the opinlon advanced by Willis (10) that cystic changes appearing in association with cancer of the lung can be secondary in regard to the tumour, i.e. results from the infective complications.

SUMMARY

Bronchopneumonic abscesses in the rat can be healed by epithelisation proceeding from the bronchi emerging into them. The epithelium lining the cavities thus formed can be of the indifferent, cuboidal, cylindric or squamous variety, obviously depending on conditions under which the healing takes place.

Epithelisation of pulmonary abscesses can be considered as one way of inducing »bronchiectasis» in the rat (and in man).

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FROM THE FIRST SURGICAL UNIVERSITY CLINIC, HELSINKI, AND FROM THE RESEARCH LABORATORY OF »ORION» PHARMACEUTICAL MANUFACTURERS

ABSORPTION AND HAEMOSTATIC ACTION OF DENATURED GELATIN SPONGE IN RABBITS

By

S. J. VIIKARI and TEUVO LARMI

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Surgical haemostasis may in most cases be effected with the aid of ordinary ligatures or tampons. There are, however, occasions when the above means are not well suited; in particular, if one aims at saving organs of vital importance in which diffuse venous or capillary bleeding is going on.

The use of suitable haemostatics has been a topic of considerable interest for several decades. The compound to be used has to fulfill three conditions. It must effect haemostasis; on the other hand, the substance must be easily absorbed by the organism, and, in addition, it must be strictly sterile.

Depending on the type of compound used, the haemostatics can be divided into two groups:

1. Substances taking part in the *coagulation*-process: thrombine (7, 8), thrombokinase (3).

2. Solids acting as *tampons:* fibrin (5), oxidized cellulose (4), gelatin (2).

It is assumed that a gelatin sponge acts as a haemostatic essentially through its tampon effect. As it stops the bleeding, the mechanism of coagulation finds more favourable conditions. It is evident that the platelets coming into the meshes of the sponge are dissolved and release thrombokinase. In the presence of thrombokinase and calcium, the prothrombin of blood plasma is changed into thrombin. Thrombin again converts fibrinogen into fibrin,

and the sponge adhering to the site of the bleeding together with fibrin stops the haemorrhage. It is just possible that a protein-coagulating substance, formaldehyde, contributes to the coagulation of blood.

Reports on the absorption of gelatin sponge have been published (6, 1). The general finding has been that the sponge is absorbed within the course of 3 to 10 weeks. The American preparation» Gelfoam» and the Danish »Spongostan» are examples of commercial products at present available.

The purpose of the present work was to study, on experimental animals, the absorption and the haemostatic action of a preparation of denatured gelatin sponge as made with the aid of a certain process, at the Research Laboratory of Orion, Pharmaceutical Manufacturers. The gelatin used was ordinary commercial gelatin plate. It was denatured by using ½ *and 1 per cent formalin. 2 per cent sodium layrylsulphonate* was added in order to promote foaming. The necessary porosity was obtained by beating the molten gelatin. After congelation the compound was sterilized in dry heat. The bacterial cultures proved negative.

METHOD

Rabbits were used as experimental animals. They were anaesthetized with ether. An incision was made in the thigh of the rabbit, and a piece of denatured gelatin was placed both in a split muscle and in the interstice between muscle and fascia; the size of the piece was 5 × 8 × 10 mm. A laparotomy was made at the same time. Pieces of 2 × 3 cm was cut out of both the liver and the omentum and a fairly intense, continuous venous and arterial bleeding was ensued. The bleeding was stopped with the aid of gelatin sponge, without any other means. The operation wound was closed. After suitable intervals, biopsy samples were taken from the regions operated, in order to study the absorption of the gelatin sponge. All the experimental animals (10 rabbits) showed a normal recovery.

The histological preparations were stained with haematoxylin-eosin; partly also by using the May-Grünwald-Giemsa and orcein stains. In an examination of the slides, attention was paid to the following factors: How long a time the network of gelatin can be

observed; what kind of cells appear in the meshes of the gelatin; what kind of cells appear at the border of gelatin and tissue; the chronological development of the symptoms.

RESULTS

After 5 days: The implant of gelatin in the muscle was firmly fixed, slightly reduced in size, but still distinctly observable. The sponge in the interstice between muscle and fascia was covered by a thin membrane, homogeneous, and reduced to about a half of its original size. The sponge in the liver incision adhered to its surroundings. It was covered by a membrane of fibrin. No signs of a post-operative haemorrhage or of infection were observable.

The *histological* examination revealed a network of gelatin, and those of its meshes which were farther from the surrounding tissue contained plenty of erythrocytes. Numerous polymorphonuclear leukocytes and macrophages could be seen in the outer part of the piece. The changes were continuous on the side of the tissue for a short distance only, without having effected any visible changes in the tissue cells themselves. The pieces of gelatin implanted between muscle and fascia and within the muscle had produced more pronounced changes than the piece in the liver. Moreover, the meshes of the sponge in the liver were almost empty (Fig. 1).

After 10 days: In the muscle and in the interstice between muscle and fascia, the sponge was still firmly fixed, further reduced, and very porous. The piece of sponge adhering to the liver was distinctly observable, being covered by adhesions. The surrounding membrane had further thickened. A *histological* examination showed an increased infiltration of the gelatin network by polymorphonuclear leukocytes and macrophages. A few giant cells were to be found close to the border of the tissue. The surrounding connective tissue had increased. In the liver, there was a great amount of connective tissue round the implant, the latter was in a close capsule, and giant cells occurred in great numbers. In other words, the picture was that of a typical foreign body reaction. The meshes of the network in the liver were still almost empty (Fig. 2).

After 20 days: The sponge in the muscle could be observed macroscopically only as a small scar. In the muscle-fascia interstice, soft remnants of the sponge could also be found. In the liver incision, the sponge could not any more be observed. The piece in the omentum had entirely disappeared. In the *histological* samples the changes were almost the same as 10 days before. Signs of a mild inflammation were observable in the muscle-fascia interstice and in the muscle.

After 30 days: Peritoneal adhesions and a small scar-like formation were observable on the liver at the site of the implantation. No spongiform material could be discovered. At a *histological* examination of the liver, no network of gelatin was observable. It was replaced by a distinct granuloma

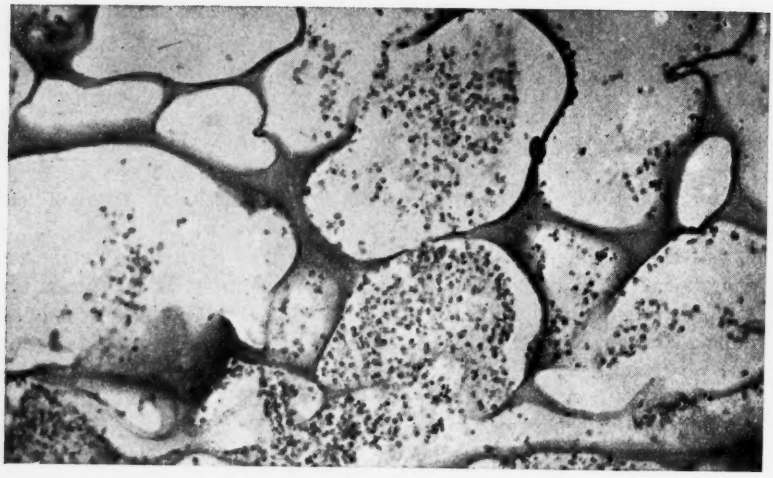

Fig. 1. — After 5 days the pores of the gelatine sponge are still clearly visible. Erythrocytes and polymorphonuclear leukocytes are to be seen in the meshes. × 130.

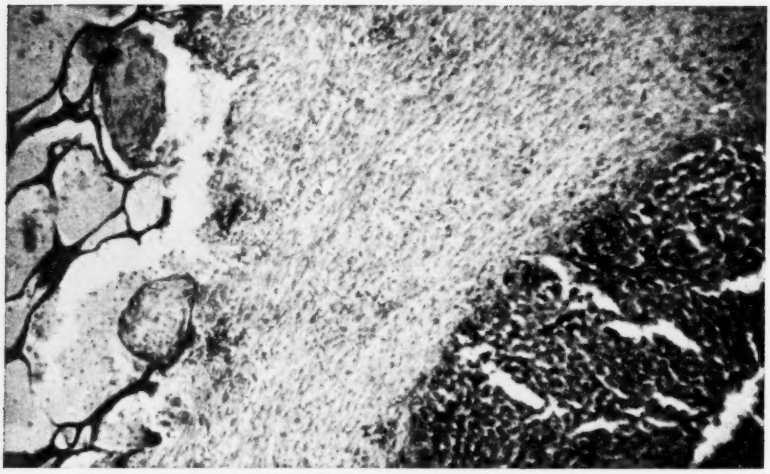

Fig. 2. — Gelatine sponge on the surface of the liver incision, after 10 days. Abundant development of connective tissue has occurred between the liver and the sponge. Giant cells and macrophages are observed at the border. × 130.

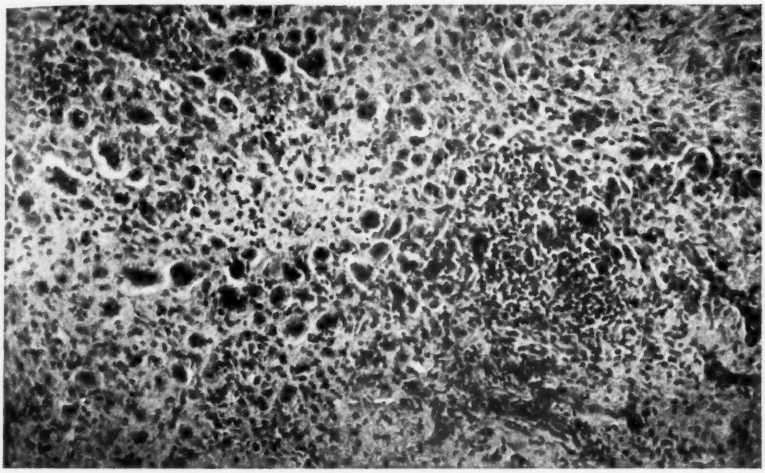

Fig. 3. — The region of the liver incision after 30 days. Gelatin is no more observable. The connective tissue has increased, and quite a number of giant cells are observable in it. Small cellular infiltrates are visible around the granuloma. × 130.

and by connective tissue. The granuloma contained numerous giant cells which included yellow granules, probably pigment arising from the disintegration products of blood. The granuloma was surrounded by some small cellular infiltrates, but these did not extend any farther into the liver tissue (Fig. 3).

After 40 days: A small scar was visible in the muscle-fascia interstice. No spongiform material could be seen either here or in the muscle. The *histological* examination revealed a granuloma in the muscle, still showing remnants of the gelatin network. Its pores contained newly formed connective tissue. The granuloma was within a closed capsule.

After 70 days: No signs of the sponge were visible at the sites of the implantation. The tissue was in these areas slightly firmer than in the surroundings. A *histological* examination did not reveal any gelatin in the tissues. Only a scar of connective tissue was visible microscopically at the sites of the implantation. In the neighbourhood of the scar of the piece placed in the muscle-fascia interstice, perivascular lymphocyte infiltration was to be seen, and in the liver, round-cell infiltration was observable surrounding the bile capillaries in the neighbourhood of the scar. However, the deeper tissues showed no changes.

An examination of the haemostatic properties of the sponge clearly shows that at least a fairly profuse, diffuse bleeding from the surface of a parenchymatous organ could be easily checked

with it. The bleeding stopped within ½—1 min. after pressing the sponge, with a finger or with a piece of gauze, tightly against the site of the haemorrhage. No difference in the effect could be observed whether one used dry sponge or sponge first wetted in saline and then squeezed dry. Numerous small arterial haemorrhages were among the bleedings stopped.

As to the absorption of the sponge, it was found to occur more rapidly in liver and omentum than in the muscle-fascia region. The absorption had distinctly started before 5 days. After 30 days, no trace of the sponge implant could be seen in the liver and the omentum. However, a small remnant was observable in the muscle up to the 40th day. After 70 days, the absorption was complete also in the muscle.

The cellular reaction around the sponge was a typical foreign body reaction. Numerous polymorphonuclear leukocytes and macrophages were to be seen at the border of the sponge and tissue during the first week. The macrophages increased in number during the second week, and giant cells were also observed close to the border of the implant. The connective tissue around the sponge also increased. A greater number of giant cells appeared in the surroundings of the sponge between the third and the fifth week. Simultaneously, the increasing connective tissue tended to form a capsule round the sponge. After the absorption of the sponge a scar of connective tissue remained at its site. The reaction of the tissue was rather slight throughout the course of the absorption, and it did not extend far from the sponge. The erythrocytes which at the beginning filled the pores of the sponge were gradually dissolved. The disintegration products were absorbed along with the absorption of the sponge.

The results indicate that gelatin sponge denatured with the aid of formalin and sodium laurylsulphonate is rapidly absorbed in the tissues and does not induce any significant reactions in its surroundings. Its haemostatic properties are quite comparable with those of other corresponding commercial products.

SUMMARY

The authors made an experimental study on rabbits of the absorption and of the haemostatic properties of gelatin sponge denatured with the aid of ½—1 per cent formalin. A piece of gelatin sponge was implanted in the interstice between muscle and fascia, in a muscle, in the omentum and on liver. Rather considerable pieces of the liver and omentum were cut out, and the resulting diffuse haemorrhage was stopped with the aid of gelatin sponge. No postoperative bleeding was observed in a relaparotomy after 5 to 70 days. A typical foreign body reaction was found at the site of the implant, at the border of the piece and the tissue. The reaction did not extend far on the side of the organic tissue. The sponge disappeared from the region of the liver approximately within 4 weeks, and from the muscle after the 6th week. After 10 weeks, only a small scar of connective tissue remained as the only microscopical sign of the implant.

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FROM THE DEPARTMENT OF ANATOMY, UNIVERSITY OF HELSINKI

EMPTYING TIME OF THE KIDNEY PELVIS

By

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(Received for publication May 8, 1951)

The emptying of the kidney pelvis, as well as the normal and pathologic physiology of the whole kidney pelvis and of the ureter, has for years been the subject of active interest and study. However, the emptying time of the kidney pelvis has not yet been definitely determined.

Attempts have been made to measure this emptying time in normal subjects. The normal values have been compared with those obtained in different pathologic conditions. In the latter, changes have been found to occur, especially prolongation. Goldstein (3) noted that, in man, the normal kidney pelvis, empties in 3—7 minutes in a retrograde pyelogram, regardless of its capacity. But by the same method Peirson (8) obtained a normal emptying time of 3—9 minutes and stated that this time depends on the size of the renal pelvis. It should be noted that measurement of the pysiological capacity of the renal pelvis is difficult, but generally values from 5 to 12 ml. have been obtained (2, 5). In different text-books of anatomy lower results are quoted, varying from 3 to 6 ml (4, 9).

For this study the same glass models of renal pelves, about natural size, were used as had been employed earlier for my tests of the hydromechanics of the renal pelvis (7). Though the conditions differ from the physiological in that no account is taken of the peristalsis of the kidney pelvis, the resilience of its wall,

I am indebted to Assistant Professor M. J. Mustakallio for many helpful suggestions throughout this investigation.

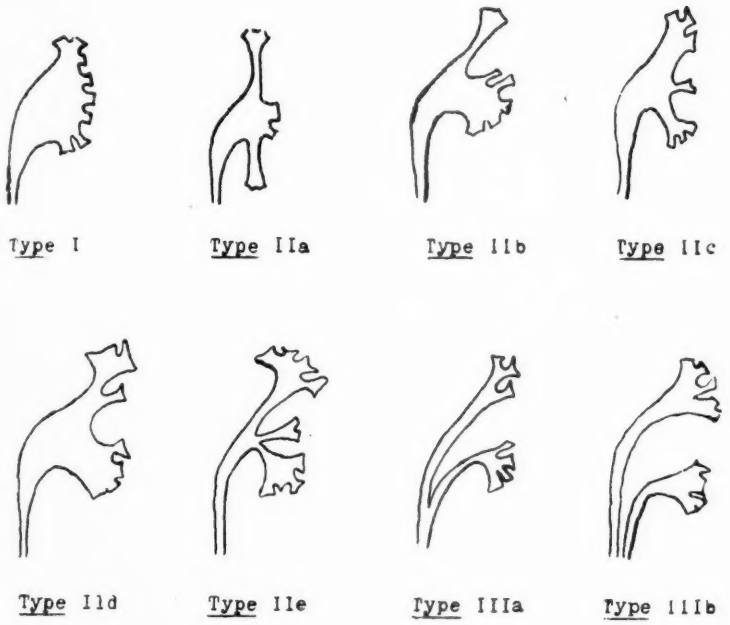

Fig. 1. — The types of renal pelvis (Jastrzebski).

and possible other factors, the figures thus obtained may be regarded in a way as fixed points in this problem.

In the tests the most common types among Jastrzebski's three main groups were used, viz. types I, IIa, IIb, IId and IIIa, which are represented in the accompanying drawing (about one-half of natural size).

The capacity of the pelves was first measured. This was done accurately by filling the glass pelvis and the short portion of ureter, with urine up to the fornices of the calices. Then the fluid was allowed to run out as far as to the ureteropelvic junction, viz. from the pelvis proper. The amount of urine that ran out then indicated the capacity of the renal pelvis. The results varied from 2.2 to 7.0 ml, with an average of 4.8 ml.

The rate of emptying was than measured for each type of glass pelvis with the aid of the device shown in fig. 2. From a glass cylinder the urine was allowed to run into the pelvis under a filtration pressure = 40 mm Hg (1), corresponding to about 53 cm urine.

With a rubber tube attached to the glass pelvis the rate of flow could be regulated. The bore of the rubber tube was 4 mm., corresponding to the inner diameter of the ureter; 1 ml then contains 15 drops of urine. Assuming the daily excretion of urine to average 1500 ml, the rate of flow was one drop per 6 seconds. Although, as has been stated, the test conditions were not physiological,

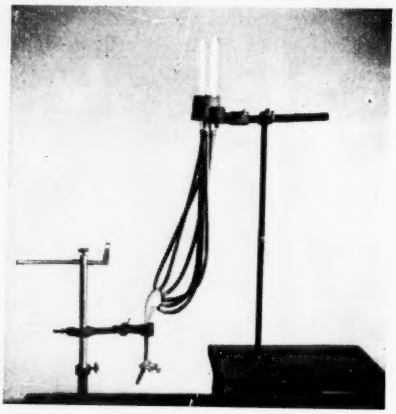

Fig. 2.

this figure corresponds with earlier ones showing that a normal pelvis empties at the rate of 1—2 ml per minute (6), viz. roughly one drop in 4 seconds.

In the following table are collected the average results obtained in tests made in the vertical position, the conditions being as outlined above. Four tests were performed with each type of pelvis.

Type	Emptying Time of Renal Pelvis	Capacity of Renal Pelvis	Emptying Time, 1 ml of Urine
I	9 min. 25 sec.	6.4 ml	1 min. 28 sec.
IIa	7 min.	3.4 ml	2 min. 3 sec.
IIb	6 min. 40 sec.	5.0 ml	1 min. 20 sec.
IId	11 min. 15 sec.	7.0 ml	1 min. 36 sec.
IIIa	6 min. 15 sec.	2.2 ml	2 min. 50 sec.

It is thus noticed that on the whole the results — varying from 6 to 11 minutes — correspond to those previously reported (6, 8). In addition, an interesting circumstance emerges when

comparing the emptying time and the capacity, viz. that the glass pelves of low capacity (especially types IIa and IIIa) empty more slowly than would be expected. This may conceivably be attributed to the fact that the capillary force in the narrow calices of these unusual types, or in the divisions of the pelvis proper, causes retardation in the outflow of fluid. It is possible that also in nature the outflow of fluid may be slower from renal pelves of this shape than from the wide and undivided types of pelvis. The emptying time may thus be said to depend, not only on the capacity of the pelvis, but als on its form.

SUMMARY

Glass models of different types of renal pelvis, about natural size, have been used for studying the emptying time of the renal pelvis. Tests performed under a given pressure (roughly 53 cm urine) and at a given rate of flow (1 drop in 6 seconds) show that the emptying time varies from 6 to 11 minutes and depends both on the capacity of the glass pelvis and on its form.

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